

FORM PTC-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE
TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY DOCKET NUMBER
CHIR-0319

U.S. APPLICATION NO. (known as) 37 C.F.R. 1.5

10/018470

INTERNATIONAL APPLICATION NO.
PCT/US00/05928

INTERNATIONAL FILING DATE
08 March 2000

PRIORITY DATE CLAIMED
30 April 1999

TITLE OF INVENTION NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

APPLICANT(S) FOR DO/EO/US Mariagrazia PIZZA, Erin HICKEY, Jeremy PETERSON, Herve TETTELIN, Craig J. VENTER, Vega MASIAGNI, Cesar GALEOTTI, Marirosa MORA, Giulio RAFFI, Maria SCARSELLI, Vincenzo SCARLATO, Rino RAPPUOLI, Claire M. FRAZER and Guido GRANDI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
- ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - A copy of the Published PCT Application by WIPO under No. WO 00/66791, including the search report.
 - A copy of the International Preliminary Examination Report.
 - Sequence listing in written (1,254 sheets) and computer readable form (3 diskettes)
 - and Statement to Support Filing and Submission.

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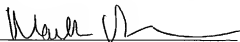
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U.S. APPLICATION NO. (if known) (37 CFR 1.53) <div style="font-size: 1.5em; font-weight: bold; margin-left: 100px;">10/018470</div>	INTERNATIONAL APPLICATION NO. CT/US00/05928	ATTORNEY DOCKET NUMBER CHIR-0319
17. The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,040.00 International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report has been prepared by the EPO or JPO..... \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$740.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00		<div style="border: 1px solid black; padding: 2px;"> CALCULATIONS PTO USE ONLY </div>
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$890.00
Surecharge of \$130.00 for furnishing the oath or declaration later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(e)).		\$
Claims	Number Filed	Number Extra
Total claims	- 20 =	X \$18.00
Independent Claims	- 3 =	x \$84.00
Multiple dependent claims(s) (if applicable)		+ \$280.00
TOTAL OF ABOVE CALCULATIONS =		\$890.00
____ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.		\$
SUBTOTAL =		\$890.00
Processing fee of \$130.00 for furnishing the English translation later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(f)).		+ \$
TOTAL NATIONAL FEE =		\$890.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property		+
TOTAL FEES ENCLOSED =		\$890.00
Amount to be:		\$
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a. <input checked="" type="checkbox"/> A check in the amount of \$890.00 to cover the above fee is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 23-3050 in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input type="checkbox"/> The Commissioner if hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.		
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.		
SEND ALL CORRESPONDENCE TO:		
Alisa A. Harbin CHIRON CORPORATION Intellectual Property - R338 P.O. Box 8097 Emeryville, California 94662-8097		
 SIGNATURE		NAME
Mark J. Rosen		NAME
39,822		REGISTRATION NUMBER

101 Rec'd PCT/PTO 21 NOV 2002
10/018470 PATENT
Atty. Dkt. No. CHIR-0319 #4
Client Dkt. No. PP0365.322

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" on **November 21, 2002** and addressed to: BOX PCT, Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Gyne Riser
Gyne Riser

11.21.02
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: **Pizza, M., et al.**

U.S. Serial No.: **10/018,470**

Int'l. Appln. No.: **PCT/US00/05928**

Group Art Unit: **not yet assigned**

Int. Filing Date: **March 8, 2000**

Examiner: **not yet assigned**

For: **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

Assistant Commissioner for Patents

Washington, D.C. 20231

BOX PCT

PRELIMINARY AMENDMENT

Applicants respectfully request entry of following amendments prior to examination on the merits, without prejudice.

In the Specification:

Please delete the Sequence Listing and insert therefore the new Sequence Listing.

In the Claims:

Please amend the claims to read as follows.

3. (Amended) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to claim 1.
5. (Amended) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to claim 1.
11. (Amended) Nucleic acid complementary to the nucleic acid of claim 7.
15. (Amended) Nucleic acid encoding a protein according to claim 6.
16. (Amended) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 8.
18. (Amended) A polyclonal or monoclonal antibody which binds to a protein according to claim 6.
19. (Amended) A nucleic acid probe comprising nucleic acid according to claim 5.
20. (Amended) An amplification primer comprising nucleic acid according to claim 5.
21. (Amended) A composition comprising nucleic acid according to claim 5.

Please cancel claims 22 and 23.

Please add the following new claims:

25. (New) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to claim 2.
26. (New) A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 25, producing an antibody which binds to the protein, and determining whether the antibody recognizes a protein produced by *N. meningitidis*.
27. (New) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to claim 2.
28. (New) A protein obtained by the method of claim 25.
29. (New) Nucleic acid encoding a protein according to claim 28.
30. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 9.
31. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 10.
32. (New) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to claim 11.
33. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 28.

- 34. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 12.
- 35. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 13.
- 36. (New) A polyclonal or monoclonal antibody which binds to a protein according to claim 14.
- 37. (New) A nucleic acid probe comprising nucleic acid according to claim 27.
- 38. (New) A nucleic acid probe comprising nucleic acid according to claim 7.
- 39. (New) A nucleic acid probe comprising nucleic acid according to claim 8.
- 40. (New) A nucleic acid probe comprising nucleic acid according to claim 9.
- 41. (New) A nucleic acid probe comprising nucleic acid according to claim 10.
- 42. (New) A nucleic acid probe comprising nucleic acid according to claim 15.
- 43. (New) A nucleic acid probe comprising nucleic acid according to claim 29.
- 44. (New) An amplification primer comprising nucleic acid according to claim 27.
- 45. (New) An amplification primer comprising nucleic acid according to claim 7.
- 46. (New) An amplification primer comprising nucleic acid according to claim 8.

47. (New) An amplification primer comprising nucleic acid according to claim 9.
48. (New) An amplification primer comprising nucleic acid according to claim 10.
49. (New) An amplification primer comprising nucleic acid according to claim 15.
50. (New) An amplification primer comprising nucleic acid according to claim 29.
51. (New) A composition comprising nucleic acid according to claim 27.
52. (New) A composition comprising nucleic acid according to claim 7.
53. (New) A composition comprising nucleic acid according to claim 8.
54. (New) A composition comprising nucleic acid according to claim 9.
55. (New) A composition comprising nucleic acid according to claim 10.
56. (New) A composition comprising nucleic acid according to claim 15.
57. (New) A composition comprising nucleic acid according to claim 29.
58. (New) A composition comprising protein according to claim 12.
59. (New) A composition comprising protein according to claim 13.
60. (New) A composition comprising protein according to claim 14.
61. (New) A composition comprising an antibody according to claim 18.

- 62. (New) A composition comprising an antibody according to claim 33.
- 63. (New) A composition comprising an antibody according to claim 34.
- 64. (New) A composition comprising an antibody according to claim 35.
- 65. (New) A composition comprising an antibody according to claim 36.
- 66. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 51.
- 67. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 52.
- 68. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 53.
- 69. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 54.
- 70. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 55.
- 71. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 56.
- 72. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 57.

73. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 58.
74. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 59.
75. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 60.
76. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 61.
77. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 62.
78. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 63.
79. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 64.
80. (New) A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 65.
81. (New) A method for detecting *N. meningitidis* antigens comprising contacting an antibody according to claim 18, 33, 34, 35, or 36 with a biological sample under conditions suitable for the formation of complexes between said antigens and said antibodies, and detecting said complexes.

PATENT
Atty. Dkt. No. CHIR-0319
Client Dkt. No. PP0365.322

82. (New) A method for detecting antibodies that selectively bind to *N. meningitidis* antigens comprising contacting a protein according to claim 6, 12, 13, 14, or 28 with a biological sample under conditions suitable for the formation of complexes between said protein and said antibodies, and detecting said complexes.

REMARKS

Applicants submit the present amendment to correct improper multiple dependencies in the claims as originally filed. New claims 25 to 82 represent subject matter from original claims 1 to 24. Support for new claims 81 and 82 is found in the specification at, for example, page 10, lines 20 to 30. No new matter has been added.

The specification has been amended to delete the Sequence Listing that was originally filed with the application and to replace it with a new Sequence Listing. The new Sequence Listing is being submitted in response to objections raised to the original Sequence Listing in the Notification of Missing Requirements. No new matter has been added.

Applicants submit that the claims are in condition for allowance, and an early Office Action to that effect is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Dated: 11/21/02

CHIRON CORPORATION
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Respectfully submitted,

By: 

Ahsa A. Harbin
Reg. No. 33,895

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please delete the Sequence Listing and insert therefore the new Sequence Listing. I

In the Claims:

Please amend the claims as follows.

3. (Amended) A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to [anyone of claims 1-2] claim 1.

5. (Amended) Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to [anyone of claims 1-2] claim 1.

11. (Amended) Nucleic acid complementary to the nucleic acid of [anyone of claims 7-10] claim 7.

15. (Amended) Nucleic acid encoding a protein according to [anyone of claims 6- 8] claim 6.

16. (Amended) A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to [anyone of claims 7-11] claim 8.

18. (Amended) A polyclonal or monoclonal antibody which binds to a protein according to [anyone of claims 12-14 or 6] claim 6.

19. (Amended) A nucleic acid probe comprising nucleic acid according to [any one of claims 5, 7-10, or 15] claim 5.

20. (Amended) An amplification primer comprising nucleic acid according to [any one of claims 5, 7-10, or 15] claim 5.

21. Amended) A composition comprising [(a)] nucleic acid according [to anyone of claims 5, 7-10, or 15; (b) protein according to anyone of claims 12-14; and/or (c) an antibody according to claim 18] claim 5.

Claims 22 and 23 have been cancelled.

New claims 25 to 82 have been added.

10/018470

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NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

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United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines*, *supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outchoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

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Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention is to provide *Neisserial* DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.



Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See ftp://ftp.tigr.org/pub/data/n_meningitidis on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least n consecutive nucleotides from the sequences and, depending on the particular sequence, n is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.

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The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

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This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psорт.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or

strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

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Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Methodology - Summary of standard procedures and techniques.

General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and II* (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference.

Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

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mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Bimstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection

(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, J. *Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, *Gibberellins: in: Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not

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readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's spliceosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,

Ranunculus, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its own set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

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into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlaskin et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha) α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polypeptide may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polypeptides or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

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protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μ m in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

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fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghirayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the

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coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with CaCl_2 or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColEI-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

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be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCIV1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be

either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guilliermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this

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invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

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labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

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advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

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Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and th-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a

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larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); (3) saponin adjuvants, such as StimulonTM (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

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individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No1 VR-590), Kirsten, Harvey Sarcoma Virus and

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Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

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D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakfield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

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WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86; Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and Nature (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

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ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

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further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

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granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of *Plasmodium falciparum* known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

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Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These

lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (supra); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biochem* 55:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as Φ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

Immunodiagnostic Assays

Neisseria MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridization

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4(\%(G + C)) - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch})$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

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In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so

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a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

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used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

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application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (e.g. to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [see also R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et. al, Nature 390, 580-586 (1997); J.-F. Tomb, et. al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

Chromosomal DNA Preparation

N. meningitidis strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were

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lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one CHCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGATCCCATATG</u>	(<i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	(<i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	(<i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	(<i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCCATATGGCCATGG</u>	(<i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	(<i>Bam</i> HI)

Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*)

3'-end primer tail: CGGGATCC (*BamHI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml $\text{NH}_4\text{-OH}$, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100µl or 1ml of water. OD_{260} was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/µl.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA→ATT; TCG→TCT; CAG→CAA; AAG→AAA; GAG→GAA; CGA and CGG→CGC; GGG→GGC.

Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 µM of each oligo, 400-800 µM dNTPs solution, 1x PCR

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buffer (including 1.5 mM MgCl₂), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

NdeI/XhoI or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

BamHI/XhoI or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

Cloning

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

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In order to introduce the recombinant plasmid in a suitable strain, 100 μ l *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800 μ l LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200 μ l of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100 μ g/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30 μ l. 5 μ l of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

Cloning

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E. coli* host W3110.

Expression

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1 μ l of each construct was used to transform 30 μ l of *E. coli* BL21 (pGEX vector), *E. coli* TOP 10 (pTRC vector) or *E. coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E. coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100 μ g/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100 μ g/ml) in 100 ml flasks, making sure that the OD₆₀₀ ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for

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pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD₂₈₀ of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer 10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M") (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

His-fusion soluble proteins large-scale purification.

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150μl Ni²⁺-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700μl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D₂₈₀ was 0.1. 21μl of each fraction were loaded on a 12% SDS gel.

His-fusion insoluble proteins large-scale purification.

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

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and thawed twice and centrifuged again. The supernatant was stored at -20°C , while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150 μl Ni^{2+} -resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C . The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD_{280} of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D_{280} of 0.02-0.06. The His-fusion protein was eluted by addition of 700 μl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD_{280} was 0.1. 21 μl of each fraction were loaded on a 12% SDS gel.

His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20 $\mu\text{g}/\text{ml}$ using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C . Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

Mice immunisations

20 μg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with $\text{Al}(\text{OH})_3$ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other

ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

ELISA assay (sera analysis)

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H₂O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA was considered positive when OD₄₉₀ was 2.5 times the respective pre-immune sera.

FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

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OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN₃) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Threshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

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Whole Extracts preparation

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

Western blotting

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

Bactericidal assay

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD₆₂₀ was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD₆₂₀ of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 2μl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

Localization of the ORFs

ORF: contig:

279 gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:
m279.seq

```

1  ATAACGCGGA  TTGCGGCTG  CTGATTTC  ACGGTTTCA  GGGCTTCGGC
51  AAGTTTGTG  GCGGCGGTT  TCATCAGGCT  GCAATGGAA  GGTACGGACA
101  CGGCGAGCG  CAGGCGCGT  TTGGCAGCG  CTCTTTGGC  GCGAGCCATG
151  GCGCGTCCGA  CGGCGCGCG  GTTGGCTGCA  ATCAGCATTT  GTCCGGTGA
201  GTTGAAGTTG  ACGGCTTCGA  CCACTTCGCT  TTGGCGGCT  TCGGCACA
251  TGGCTTTAAC  CTGCTCATCT  TCCAAGCCGA  GAATCGCGC  CATTGCGCC
301  ACGGCTTGG  GTACGGCGGA  CTGCATCAGT  TCGGCGCGCA  GCGCACGAG
351  TTTGACCGCG  TCGGCCAAAT  TCAATGCGCC  GCGGCAACG  AGTGGGTGT
401  ATTGCGCGAG  GCTGTGTCCG  GCAACGGCG  CAGGCGTTT  GCGGCCGCT
451  TCTAAATAG

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This corresponds to the amino acid sequence <SEQ ID 3; ORF 279>:

m279.pep

```

1  ITRICGCLIS TVFRASASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAM
51  ARPTAALPA ITTCPELKL TASTTSLWAA SAQMALTCSS SKPMAAIAP
101 TPCGTADCS SARRRSLTA SAKFNAPAAT SAVISPRLCP ATAAGVLPPA
151 SK*

```

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 4>:

g279.seq

```

1  atgacgcgga ttgcgggctg cttgatttca acggttttga gtgtttcggc
51  aagttttgtc ggcgggggtt tcatcaggct gcaatgggaa ggaacggata
101  ccggcagcgg caggcgcggt ttgcttcggg cttctttggc ggcagccatg
151  gtgcgtcoga cggcgggcgc gttgcctgca atcacgactt gtccgggcga
201  gtgaaagtgc acggttttga ccaacttgcg ctgtgcgcat tgggcacaaa
251  tgcgcttgac ctgttcattc tccaaaccca aaatggcgcg cattgcgcct
301  acgcttcgca ctgacggcga ctgpatcagt tcggcgcgca ggcggcagag
351  tttagacgga tcggcgaagt ccaatgtctc ggcggcgaca agcgcggtgt
401  attcgcgag gcgtgtcggc gcaacgcggc caggcgtttt ggcgcgccat
451  tccaaatag

```

This corresponds to the amino acid sequence <SEQ ID 5; ORF 279.ng>:

g279.pep

```

1  MTRICGCLIS TVLSVASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAM
51  VRPTAALPA ITTCPELKL TASTTSPCAD SAQICLTCS SKPMAAIAP
101 TPCGTADCS SARRRSLTA SAKNSAAPT SAVISPRLCP ATAAGVLPP
151 SK*

```

ORF 279 shows 89.5% identity over a 152 aa overlap with a predicted ORF (ORF 279.ng) from *N. gonorrhoeae*:

	10	20	30	40	50	60
m279.pep	ITRICGCLISTVFRASASLSAAGFIRLOWEGTDTGSGRARLAPASLAAMARPTAALPA					
g279	MTRICGCLISTVLSVASLSAAGFIRLOWEGTDTGSGRARLAPASLAAMVRPTAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITICPELKLTA TSTTSLWASAAQMALTCSSSKPMAAIAPTPCGTADCISSARRRSLTA					
g279	ITTCPELKLTA TSTTSPCADSAQICLTCS SKPMAAIAPTPCGTADCISSARRRSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKFNAPAATSAVISPRLCPATAAGVLPPASKX					
g279	SAKNSAATSAVISPRLCPATAAGVLPPPTSKX					
	130	140	150			

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 6>:

a279.seq

```

1  ATGACNCNGA TTGCGGGCTG CTTGATTCCA ACGGTTTNNa GGGCTTCGGC
51  GAGTTTGTGC GCGGCGGGTT TCATGAGGCT GCAATGGGAA GGTACNGACA
101  CNGGCAGCGG CAGGCGCGGT TTGCGCGCGG CTTCTTTGGC GGCAAGCATA
151  GCGCGCTCGA CCGCGCGCGG ATTGCCTGCA ATCACGACTT TTCGCGCGCA
201  GTTGAAGTTG ACAGCTTCAA CCACTTCATC CTGTGCGGAT TCGGCGCAAA
251  TTGTATTAC CTGTTCATCT TCCAAGCGGA GAATCGCGCG CATTGCGCCC
301  ACAGCTTCGC GTACGCGCGA CTGCATCGT TCGGCGCGCA NGCGCACGAG
351  TTGACGCGG TCGGCAAAAT CCAATGCGCG GCGGCGACN AGTGCAGTGT

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401 ATTCGCGCAN GCTGTGTCCG GCAACGGCGG CAGCGCTTT GCCGCCGCT
451 TCCGAATAG

This corresponds to the amino acid sequence <SEQ ID 7; ORF 279.a>:

a279.pep
1 MTXICGCLIS TVXRASASLS AAGFMRLQWE GTDTGSGRAR LAPASLAASI
51 ARSTAAALPA ITTCPGELKL TASTSSCAD SAQICFTCS SKPRIAATAP
101 TPCGTADCI SARKXRTSLTA SAKSNAPRAAT SAVVSEKLCF ATAAGVLPAA
151 SE*

m279/a279 ORFs 279 and 279.a showed a 88.2% identity in 152 aa overlap

	10	20	30	40	50	60
m279.pep	ITRICGCLISTVFRASASLSAAGFIRLOWEGTDTGSGRARLAPASLAARHAPRTAAALPA					
a279	MTXICGCLISTVXRASASLSAAGFMRLQWE GTDTGSGRARLAPASLAASARSTAAALPA					
	10	20	30	40	50	60
	70	80	90	100	110	120
m279.pep	ITTCPGELKLTA STTSSLAWSAQMALTCSSSKPRIAATPTPCGTADCISSARRRTSLTA					
a279	ITTCPGELKLTA STTSSCADSAQICFTCS SKPRIAATPTPCGTADCISSARRRTSLTA					
	70	80	90	100	110	120
	130	140	150			
m279.pep	SAKFNAPRAATSAVYSPRLCPATAAGVLPASKX					
a279	SAKSNAPRAATSAVYSPKLCPTATAAGVLPASEX					
	130	140	150			

519 and 519-1 gnm7.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 8>:

m519.seq (partial)
1 TCCGTTATCG GCGGTATGGA GTTGGACAAA ACGTTTGAAG AACGCGACGA
51 AATCAACAGT ACTGTTGTTG CCGCTTTGGA CGAGGCGGCC GGCGTTTGG
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGGTTCC GCCGCAAGAA
151 ATCCTTCGCT CAATCGAGCG GCAAAATTAAT GCCGAACGCG AAAAAGCCGC
201 CCGTATCGCC GAATCCGAAG GTGCTAAAT CGAACAAATC AACCTTGCCA
251 GTGGTCAGCG CGAAGCCGAA ATCCAACAAT CCGAAGCGGA GCCTCAGGCT
301 GCGGTCAAGT CGTCAATATG CGAGAAAATC GCCCGCATCA ACCGCGCCAA
351 AGGTGAAGCG GAATCCTTGC GCCTTTGTGC CGAAGCCAAT GCCGAAGCCA
401 TCGTCAAAAT TGCCGCGGCC CTTCAAAACC AAGGCGGTGC GGATGCGGTC
451 AATCTGAAGA TTGCGGAACA ATACGTGCGT CGCTTCAACA ATCTTGCCAA
501 AGAAAGCAAT ACGCTGAITA TGCCCGCCAA TGTTCGCGAC ATCGCGAGCC
551 TGATTCTCG CGGTATGAAA ATTATCGACA CGACGAAAC CGCCAAATAA

This corresponds to the amino acid sequence <SEQ ID 9; ORF 519>:

m519.pep (partial)
1 SVIGRMELDK TFEERDEINS TVVAALDEAA GAWGVKLVRY EIKDLVFPQE
51 ILRSMQAQIT AEREKRARIA ESEGRKIEQI NLAGGQREAE IQQSEGAQA
101 AVNASNAEIK ARINRKAEGA ESLRLVAEAN AEATROIAAA LQTGGADAV
151 NLKIAEYVA AFNNLAKESN TLIMPANVAD IGLSISAGMK IIDSSKATK*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 10>:

g519.seq
1 atggaatttt tcattatcct gttggcagcc gtcgcgctt tcgggtccaa
51 atcctttgtc gtcaccccc agcaggaagt ccacggtgtc gaaaggctcg

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101 ggcgtttcca tcgcgcctcg acggccggtt tgaatatatt gattcccttt
151 atcgaccgcg tcgcctaccg ccattcgctg aaagaaatcc ctttagacgt
201 acccagccag gtctgcatac cgcgcgataa tacgcaattg actgttgacg
251 gcatcatcta ttcccaagta accgatccca aactcgcttc atacggttgc
301 agcaactaca ttatggcaat taccagctt gcccaacga cgctcgcttc
351 cgttatcggg cgtatggagt tggacaaaac gtttgaagaa cgcgacgaaa
401 tcaacagtac cgtcgtctcc gccctcgatg aagccgcggg ggcttggggt
451 gtgaagatcc tcggttacga aatacaggat ttggttcgcg cgcaagaata
501 ccttcgcgca atgcaggcac aaattaccgc cgaacgcgaa aaacgcgcc
551 gtattgcgca atccgaaggc cgtaaaatcg aacaaatcaa ccttgccagt
601 ggctcagctg aagccgaaat ccaacaatcc gaaggcgagg ctcaggctgc
651 ggctcaatgc tccaatgcc agaaaaatcg ccgcatcaac cgcgccaaag
701 gcgaagcgga atccctgcgc cttgttgcg aagccaatgc cgaagccaac
751 cgtcaaatgt ccgccccttc tcaaacccaa agcggggcgg atcgcgtaaa
801 tctgaagatt gcgggacaa acgttaccgc gttcaaaaat cttgcgaag
851 aagacaatac gcgattaaag ccgcgcaagg ttgcgcaaat cgggaacct
901 aattttcgcc ggcattgaaa attttcgcca gaagcaaaaa cggccaaata
951 a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

```

g519.pep
1  MEFFILLAA VAVFGFKSFV VLPQEEVHV ERLGRPHRAL TAGNLILIFP
11 IDRVAYRHSI KEPLDVPQ VCITRDNTQL TVDGIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLYRIKID LVPPQELIRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAAQAVNA SNAEKIARIN RAKGEAESLR LVAEANAEN
251 RQIAAALQTS SGADAVNLKI AGQYVTAFFN LAKEDNTRIK PAKVAEIGNP
301 NFRHEKFSP EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

```

m519/g519
m519.pep                                     10          20          30
                                      SVIGRMELDKTFEERDRINSTVVAALDEAA
g519      YFQVTPDKLASYGSSNYIMAITQLAQTTLSVIGRMELDKTFEERDRINSTVVSALDEAA
          90          100          110          120          130          140

m519.pep          40          50          60          70          80          90
GAWGVKVLRYEIKDLVPPQELIRMQAQITAEREKRARIAESEGRKIEQINLASGGQREAE
g519      GAWGVKVLRYEIKDLVPPQELIRMQAQITAEREKRARIAESEGRKIEQINLASGGQREAE
          150          160          170          180          190          200

m519.pep          100          110          120          130          140          150
IQOSEGEAAQAVNASNAEKIARINRAKGEAESLRILVAEANAENRQIAAALQTSQGADAV
g519      IQOSEGEAAQAVNASNAEKIARINRAKGEAESLRILVAEANAENRQIAAALQTSQGADAV
          210          220          230          240          250          260

m519.pep          160          170          180          190          200
NLKIAEQYVAAPFNLLAKESNTLIMPANVADIGSL-ISAGMKIIDSSKTAK
g519      NLKIAEQYVTAFFNLAKEDNTRIKPAKVAEIGNPFRHEKFSP EAKTAK
          270          280          290          300          310

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq


```

1 ATGGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51 ATCCTTTGTT GTCAATCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GGGGTTTCCA TCGCGCCCTG ACGGCGCGGT TGAATATTTT GATTCCTTTT
151 ATCGACGCGG TCGGCTACCG CCATTCCGTC AAGAATATCC CTTTAGACGT
201 ACCGAGCCAG GTCTGATCA CCGCGGACAA TACGAGCTCG ACTGTTGACG
251 GTATCATCTA TTTCGAAGTA ACCGACCCCA AACTCGGCTC ATACGGTTTC
301 AGCAATCTACA TTATGCGCAT TACCCAGCTT GCCCAACAGA GCGTCGCTTC
351 CTTATCGGGG COTATGGAT TGGACAAAC GTTTGAGAA CCGACGAA
401 TCACAGACAG CCTCGCTCC GCCTCGATG AAGCGCGCG AGCTTGGGGT
451 GTGAAGGTTT TCGGTTATGA GATTAAGAC TTGGTTCCGG CGCAAGAAAT
501 CCTTCGCTCA ATGCGAGGCG AATTAATGCG TGAACGGGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAATGCG AACAAATCAA CCTTGCCAGT
601 GGTGAGCGCG AAGCGGAAAT CCAACAATCC GAAGCGGAGG CTCAGGCTGC
651 GGTCAATGCG TCAATGCGCG AGAAATGCG CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGGCG CTTGTTGCGG AAGCCAATCG CGAAGCCATC
751 CGTCAAATTC CCGCGCGCTT TCAACCCCA GCGGCTGCGG ATCGGCTCAA
801 TCTGAAGATT GCGGAACAT ACCTGCGCG GTTCACAAAT CTGCGCAAG
851 AAGCAATAC GCTGATTATG CCGCCAATG TTGCGGACAT CGGAGCCTG
901 ATTTCTGCGG GTATGAAAT TATCGACAG AGCAAAACCG CCAATAAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1 MEFFIILLAA VVVEGFKSFV VIPQEVHVHV ERLGRFHRAL TAGLNILIPF
51 IDRVAIRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIFYOV TDPKLAISYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAG
151 VKVLRYEIKD LVPPQEIILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAIQGS ECEAQAQVNA SNAEKIARIN RAKGEAESLR LVAEANAELI
251 RQIAALQQT GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

m519/a519 ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

m519.pep
10 20 30
SVIGRMELDKTFEERDEINSTVVAALDEAA
a519 YFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVVSALDEAA
90 100 110 120 130 140

m519.pep
40 50 60 70 80 90
GAWGVKVLRYEIKDLVPPQEIILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAE
a519 GAWGVKVLRYEIKDLVPPQEIILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAE
150 160 170 180 190 200

m519.pep
100 110 120 130 140 150
IQQSEGEAQAQVNASNAEKIARINRAKGEAESLRLVAEANAELIRQIAALQQTGGADAV
a519 IQQSEGEAQAQVNASNAEKIARINRAKGEAESLRLVAEANAELIRQIAALQQTGGADAV
210 220 230 240 250 260

m519.pep
160 170 180 190 200
NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSTAKX
a519 NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSTAKX
270 280 290 300 310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTGCGCGTTT TCGGTTTCAA
51  ATCCTTTGTT GTCATCCCCC AACAGGAAGT CCACGTTGTC GAAAGCGCTG
101 GCGGTTTCCA TCGCGCCCTG ACGGC CGGTT TGAATATTTT GATTCCTTTT
151 ATCGACGCGG TCGCCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201 ACCGAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGAGC
251 GCATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGTTGCG
301 AGCACTACGA TTATGGGAT TACCCAGCTT GCCCAAACGA CGTCGGCTTC
351 CTTTATCGCG GGTATGGAGT TGGACAACG GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC TGTGTTGGCG GCTTTGGAGC AGCGCGCCGG GCCTTGGGCT
451 GTGAAGGTTT TGCCTTATGA GATTAAAGAC TTGTTCCCG CGCAAGAAAT
501 CTTTCGCTCA ATGCAGGCGC AAATTACTGC CGAACGCGAA AAACGCGCCC
551 GTATCGCGCA ATCCGAAGGT CGTAAATCG AACAAATCAA CTTGCCAGT
601 GTTCAGCGCG AAGCGGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAATGCGC AGAAAATCG CGCATCAAC CGCGCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCGC AAGCCAATGC CGAAGCCATC
751 CTTCAATTCG CCGCGCCCTT TCAACCCAA GCGCGTGGGG ATGCGGTCAA
801 TCTGAAGATT CGGAAACAT ACCTGCTGCG GTTCAACAT CTTCGCAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCGACAT CGGCAAGG
901 ATTTCTGCGG GTATGAAAT TATCGACAGC AGCAAAACCG CCAATAA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVFGFKSFV VIFQOEHVHV ERLGRFHRAL TAGNIIILIF
51  IDRVAYRHS LKEIPLDVPSQ VCITRDNTQL TVDGIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151 VKVLRYEIKD LVFPQELIRS MQAQITAEER KRARIAESER RKIEQINLAS
201 GOREAEIQGS EGGAAQAVNA SNAEKIARIN RAKGAESELR LVAEANAEAI
251 RQIAAALQIQ GGAIVNLKI AEQYVAAPFN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTGCGCGTTT TCGGTTTCAA
51  ATCCTTTGTC GTCATCCCCC AGCAGGAAGT CCACGTTGTC GAAAGCGCTG
101 GCGGTTTCCA TCGCGCCCTG ACGGC CGGTT TGAATATTTT GATTCCTTTT
151 ATCGACGCGG TCGCCTACCG CCATTGCGTG AAAGAAATCC CTTTAGACGT
201 ACCGAGCCAG GTCTGCATCA CGCGCGATAA TAGCAATTG ACTGTTGAGC
251 GCATCMCTTA TTTCCAAGTA ACGATCCCA AACTCGCCTC ATACGTTGCG
301 AGCAACTACA TTATGGGAT TACCCAGCTT GCCCAAACGA CGTCGGCTTC
351 CGTTATCGGG CTTATGGAGT TGGACAAAC GTTTGAAGAA CGCGACAAA
401 TCAACAGTAC CGTCTCTCC GCCCTCGATG AAGCGCGCGG GCCTTGGGCT
451 GTGAAAGTCC TCGGTTACGA AATCAAGGAT TTGTTCCCG CGCAAGAAAT
501 CTTTCGCGCA ATGCAGGCAC AAATTACCG CGAACGCGAA AAACGCGCCC
551 GTATTGCGCA ATCCGAAGGC CGTAAATCG AACAAATCAA CTTGCCAGT
601 GGTCAAGCTG AAGCGGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAATGCGC AGAAAATCG CGCATCAAC CGCGCAAAG
701 GCGAAGCGGA ATCCCTGCGC CTTGTTGCGC AAGCCAATGC CGAAGCCATC
751 CTTCAATTCG CCGCGCCCTT TCAACCCAA GCGCGGCGCG ATGCGGTCAA
801 TCTGAAGATT CGGAAACAT ACCTGCTGCG GTTCAACAT CTTCGCAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCGACAT CGGCGCTG
901 ATTTCTGCGG GCATGAAAT TATCGACAGC AGCAAAACCG CCAATAA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVFGFKSFV VIFQOEHVHV ERLGRFHRAL TAGNIIILIF
51  IDRVAYRHS LKEIPLDVPSQ VCITRDNTQL TVDGIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVFPQELIRS MQAQITAEER KRARIAESER RKIEQINLAS
201 GOREAEIQGS EGGAAQAVNA SNAEKIARIN RAKGAESELR LVAEANAEAI
251 RQIAAALQIQ GGAIVNLKI AEQYVAAPFN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

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m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
g519-1.pep	MEFFIIILAAVAVFGFKSFVVP	IQQEVHVVERLGRFHRALT	AGLNLIFPIDRVARHSL			
m519-1	MEFFIIILVAVAVFGFKSFVVP	IQQEVHVVERLGRFHRALT	AGLNLIFPIDRVARHSL			
	70	80	90	100	110	120
g519-1.pep	KEIPLDVPSQVCITRDNTQLT	VDGIIYFQVTDPKLASYGSS	NYIMAITQLAQTTLSRIG			
m519-1	KEIPLDVPSQVCITRDNTQLT	VDGIIYFQVTDPKLASYGSS	NYIMAITQLAQTTLSRIG			
	130	140	150	160	170	180
g519-1.pep	RMELDKTFEERDEINSTVVS	ALDEAAGANGVKVLRYEIK	DLVPPQEILRAMQAQITAE			
m519-1	RMELDKTFEERDEINSTVVS	ALDEAAGANGVKVLRYEIK	DLVPPQEILRSMAQITAE			
	190	200	210	220	230	240
g519-1.pep	KRARIAESGRKIEQINLASG	QREAEIQQSEGEAAQAVN	ASNAEKIRINRAKGEAESL			
m519-1	KRARIAESGRKIEQINLASG	QREAEIQQSEGEAAQAVN	ASNAEKIRINRAKGEAESL			
	250	260	270	280	290	300
g519-1.pep	LVAEANAIAIRQIAAALQT	GGGADAVNLKIAEQYVAA	FNNLAKESNTLIMPANVAD	IGSL		
m519-1	LVAEANAIAIRQIAAALQT	GGGADAVNLKIAEQYVAA	FNNLAKESNTLIMPANVAD	IGSL		
	310					
g519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

a519-1.seq

1	ATGGCAATTTT	TCATTATCTT	GCTGGCAGCC	GTCGTTGTTT	TCGGCTTCAA
51	ATCCCTTTGTT	GTCATCCCCAC	AGCAGGAAGT	CCACGTTGTC	GAAGGCGTCG
101	GGCGTTTCCA	TCCGCGCCTG	ACGCGCGGTT	TGATATTTTT	GATTCGCTTT
151	ATCGACCGCG	TCCGCTACCG	CCATTTCGCT	AAGAAATCC	CTTTACGCT
201	ACCCAGCCAG	GTCTGCATCA	CSCCGGACAA	TACGAGCTG	ACTGTTGACG
251	GTATCATCTA	TTTCCAAGTA	ACGACCCCA	AACTCGCTC	ATACGGTTTCG
301	AGCAACTACA	TTATGGCGAT	TACCCAGCTT	GCCCAAACGA	CGCTCGGTTT
351	CGTTATCGGG	CGTATGGAAT	TGGACAAAAC	GTTTGAAGAA	CGCGACGAAA
401	TCAACAGCAC	CGTCGCTCC	CGCCTCGATG	AAGCCGCGGG	AGCTTGGGGT
451	GTGAAGGITT	TGCGTTATGA	GATTAAAGAC	TTGGTTCGCG	CGCAAGAAAT
501	CTTCGCTCA	ATCGAGCGGC	AAATTACTCG	TGAACGCGAA	AAACGGCGCC
551	GTATCGCCA	ATCCGAAGGT	CGTAAATCG	ACAAATCAA	CTTCCGAGT
601	GGTCAGCGCG	AAGCCGAAT	CCAAATCC	GAAGCGAGG	CTCAGCGTGC
651	GGTCAATGCG	TCAATGCCG	AGAAATCGC	CGCATCAAC	CGCGCCNAG
701	GTGAAGCGGA	ATCCTTGCGC	CTTGTGCGC	AAGCCAATGC	CGAAGCCATC
751	CGTCAAAATG	CCGCCGCCCT	TCAAACCCAA	GGCGGTGCGG	ATGCGGTCAA
801	TTCTGAAGATT	CGGGAACAAT	ACGTGCGCGC	GTTCACAAT	CTTGCCAAAG
851	AAAGCAATAC	GCTGATTATG	CCGCCCAATG	TTGCCGACAT	CGGCAGCCTG
901	ATTTCTGCGG	GTATGAAAT	TATCGACAGC	AGCAAAACCG	CCAAATAA

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This corresponds to the amino acid sequence <SEQ ID 19; ORF 519-1.a>:

a519-1.pep.

```

1  MEFFIILLAA VVVEGFKSFV VVPOQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHS
51  IDRVAYRHS KEIPLDVP SQ VCITRDNTOL TVDGIIFYQV TOPKLASYGS
101 SNYIMAITOL AQTTLR SVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVFPQEI LRS MQAQITAE RE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQA VNA SNAEKIARIN RAKGEAESLR LVAEANAEAI
251 RQIAAALQTO GGADAVNL KI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa overlap

```

              10      20      30      40      50      60
a519-1.pep  MEFFIILLAAVVVFGFKSFVVIPOQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHS
              10      20      30      40      50      60
m519-1      MEFFIILLVAVAVFGFKSFVVIPOQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHS
              10      20      30      40      50      60
              70      80      90      100     110     120
a519-1.pep  KEIPLDVP SQVCITRDNTOLTVDGIIFYQVTDPKLASVGS SNYIMAITOLAQTTLRSVIG
              70      80      90      100     110     120
m519-1      KEIPLDVP SQVCITRDNTOLTVDGIIFYQVTDPKLASVGS SNYIMAITOLAQTTLRSVIG
              70      80      90      100     110     120
              130     140     150     160     170     180
a519-1.pep  RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVFPQEI LRS MQAQITAE RE
              130     140     150     160     170     180
m519-1      RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVFPQEI LRS MQAQITAE RE
              130     140     150     160     170     180
              190     200     210     220     230     240
a519-1.pep  KRARIAESEG RKIEQINLASGQREAEIQOSEGEAQA VNASNAEKIARINRAKGEAESLR
              190     200     210     220     230     240
m519-1      KRARIAESEG RKIEQINLASGQREAEIQOSEGEAQA VNASNAEKIARINRAKGEAESLR
              190     200     210     220     230     240
              250     260     270     280     290     300
a519-1.pep  LVAEANAEAI RQIAAALQTOGGADAVNL KIAEQYVAAFNNLAKESNTLIM PANVADIGSL
              250     260     270     280     290     300
m519-1      LVAEANAEAI RQIAAALQTOGGADAVNL KIAEQYVAAFNNLAKESNTLIM PANVADIGSL
              250     260     270     280     290     300
              310
a519-1.pep  ISAGMKIIDSSKTAKX
              310
m519-1      ISAGMKIIDSSKTAKX
              310

```

576 and 576-1

gnum22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

m576.seq.. (partial)

```

1  ..ATGCAGCAGG CAAGCATGCG GATGGGCGTG GACATCGGAC GCTCCCTGAA
51  GCAAATGAAG GAACAGGCGC CGGAAATCGA TTTGAAAGTC TTTACCGAAG
101 CCATGCAGGC AGTGATGATG GCGAAGAGAA TCAAATGACG CGAAGAGCAG
151 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAGCCGT
201 AGAAAAACAC AAGCGCGAGC CGAAGGCCAA TAAAGAAAAA GCGCAGACCT

```

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```

251 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCGGCG
301 CTGCAATACA AAATCACCAA ACAGGGGGAA GGCRAACAGC CGACCAAAGA
351 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCGTATTGAC GGTACGGTAT
401 TCGACAGCAG CAAAGCCCAAC GCGGCCCGCG TCACCTTCCT TTTGAGCCAA
451 GTGATTTCGG GTTGACCGA AGCGCTACAG CTCTGAAAG AAGCGCGCGA
501 AGCCACGTTT TACATCCCGT CCAACTTCGG CTACCGCGAA CAGGGTCGGG
551 GCGACAAAT CGCTCGACAC GCCACTTGG TATTTGATGT GAAACTGGTC
601 AAATCGCGC CACCCGAAAA CCGGCCCGCG AAGCAGCCGG CTCAAGTCGA
651 CATCAAAAAA GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 21; ORF 576>:

```

m576.pep.. (partial)
1  ..MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEO
51  AOEVMVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
101 LOYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPFLQV
151 VIPGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVEDVKLV
201 KIGAPENAPA KQPAQVDIKK VN*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 22>:

```

g576.seq.. (partial)
1  ..atggggcgctgg acatcggcagc ctccctgaaa caaatgaagg aacaggggcgc
51  ggaatatcgat ttgaaagtct ttacogatgc catcgaggca gtgtatgacg
101 gcaagaagaat caaatgacc gaagagcagg cccaggaagt gatgatgaaa
151 ttcctgcagg agcagcaggc taaagccgta gaaaaacaca aggcggatgc
201 gaaggccaac aagaaagaag gcgaaggcct ctgaaggaa aatgcgcgcg
251 aagacggcgt gaagaccact gcttcggctc tgcagtaca aatcaccaaa
301 cagggtgaag gcaaacagcc gacaaaagac gacatcgta ccgtggaata
351 cgaagggcgc ctgattgacg gtacogtatt cgacagcagc aaagccaacg
401 gcggcccggc cacccttcct ttgagccaag tgattccggg ttggaccgaa
451 ggcgtacggc tctgaaaga aggcggcgaa gccacgttct acatcccgtc
501 caaccttgcc taccgcgaac agggcgagg cgaaaaaatc ggtccgaacg
551 ccaacttggt atttgacgtg aaactggcta aaatcggcgc acccgaaaac
601 gcgcccgcga agcagccgga tcaagtcgac atcaaaaaag taaataaa

```

This corresponds to the amino acid sequence <SEQ ID 23; ORF 576.ng>:

```

g576.pep.. (partial)
1  ..MGVDIGRSLK QMKEQGAED LKVFDTAMQA VYDGKEIKMT EEQAQEVMMK
51  FLOEQQAKAV EKHKADAKAN KEKGEAFLE NAAEDGVKTT ASGLQYKITK
101 QGEGKQPTKD DIVTVEYER LIDGTVEFSS KANGGPATFP LSOVIPGWTE
151 GVRLLKEGGE ATFYIPSNLA YREQGAGEKI GPNATLVFDV KLVKIGAPEN
201 APAKQPDQVD IKKVN*

```

Computer analysis of this amino acid sequence gave the following results:

Homology with a predicted ORF from *N. gonorrhoeae*

m576/g576 97.2% identity in 215 aa overlap

```

              10      20      30      40      50      60
m576.pep  MQQASYAMVDIGRSLKQMKEQGAEIDLKVFFTEAMQAVYDGKEIKMTEEQAEQVMVMKFLQ
g576      |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
              10      20      30      40      50
m576.pep  EQQAKAVEKHKADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQEGEGKQPTKDDIV
g576      |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
              70      80      90      100     110     120
m576.pep  EQQAKAVEKHKADAKANKEKGEAFLENAAEDGVKTTASGLQYKITKQEGEGKQPTKDDIV
g576      |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
              60      70      80      90      100     110

```

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```

              130      140      150      160      170      180
m576.pep      TVEYEGRLIDGTVFDSSKANGGPFVTFPLSQVIFGWTEGVQLKEGGEATFYIPSNLAYRE
g576           TVEYEGRLIDGTVFDSSKANGGPFATFPLSQVIFGWTEGVRLLEKGEATFYIPSNLAYRE
              120      130      140      150      160      170

              190      200      210      220
m576.pep      OGAGDKIGPNATLVFDVLVKIGAFENAFAPKQPAQVDIKKVN
              |||||
g576           OGAGEKIGPNATLVFDVLVKIGAFENAFAPKQPDQVDIKKVN
              180      190      200      210

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 24>:

```

a576.seq
1   ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CGCCTTTGCG
51  ACTTTCCGCC TCGCGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCGCGCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCAGC
151 ATGCGAGCAG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAATGAGG GAACAGGGCG CGGAATTCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAAGC AGCTATGAG GCAAGAAJAA TCAAAATGAC CGAAGCAGC
301 GCTCAGGAAG TCATGATGAA ATTCTTTCAG GAACAACAGG CTAAGCCCT
351 AGAAAACAC AAGGCGGAGC CGAAGGCCAA TAAAGAAAAA GCGAAGCCT
401 TCTGAARAA AAATGCCGCC AAAGACGGCG TGAAGACCA C TGCTTCCGCG
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAACAGC CGACCAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGCGCG CCTGATTGAC GGTACGSTAT
551 TCGACAGCAG CAAAGCCAA C GCGCGCCCG TCACCTTCCC TTTGAGCCAA
601 GTGATTCTGG GTTGACCGGA AGGCGGTACG CTTCTGAAG A AAGCGCGCGA
651 AGCCACCTTC TACATCCCGT CCAACCTTTC CTACCGCGAA CAGGTCGCG
701 CGCACAAAT CGCCCGGAC CCACCTTGG TATTGATGT GAACTGGTC
751 AAAATCGGCG CACCCGAAA C GCGCCCGCC AAGCAGCGCG CTCAGTCGA
801 CATCAAAAAA GTAATTAA

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This corresponds to the amino acid sequence <SEQ ID 25; ORF 576.a>:

```

a576.pep
1   MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEFAAASSA QGDTSSIGST
51  MQQASYAMGV DIGRSLQKMQ EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMMKFLO EQQAKAVEKH KADAKANKER GEAFLENAA KDGVTITASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGKIGPN ATLFDVKLV
251 KIGAFENAPA KQPAQVDIKK VN*

m576/a576      ORFs 576 and 576.a showed a 99.5% identity in 222 aa overlap

a576.pep
              10      20      30
              MQQASYAMGV DIGRSLQKMQ EQGAEIDLKV
a576           CGKKEAAPASASEFAAASSAQGDTSSIGSTMQQQASYAMGV DIGRSLQKMQ EQGAEIDLKV
              30      40      50      60      70      80

              40      50      60      70      80      90
m576.pep      FTEAMQAVYD GKEIKMTEEQ AQEVMMKFLO EQQAKAVEKH KADAKANKER GEAFLENAA
a576           FTEAMQAVYD GKEIKMTEEQ AQEVMMKFLO EQQAKAVEKH KADAKANKER GEAFLENAA
              90      100     110     120     130     140

              100     110     120     130     140     150
m576.pep      KDGVTITASGLQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKANGGPFVTFPLSQ
a576           KDGVTITASGLQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKANGGPFVTFPLSQ
              150     160     170     180     190     200

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              160      170      180      190      200      210
m576.pep      V I P G W T E G V Q L L K E G G E A T F Y I P S N L A Y R E Q G A G D K I G P N A T L V F D V K L V K I G A P E N A P A
a576           V I L G W T E G V Q L L K E G G E A T F Y I P S N L A Y R E Q G A G D K I G P N A T L V F D V K L V K I G A P E N A P A
              210      220      230      240      250      260

              270
m576.pep      K Q P A Q V D I K K V N X
a576           I I I I I I I I I I
              K Q P A Q V D I K K V N X
              270

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 26>:

```

m576-1.seq
1  ATGAACACCA  TTTTCAAAT  CAGCGCACTG  ACCCTTTCCG  CGGCTTTGGC
51  ACTTTCCGCC  TCGGCAAAA  AAGAAGCCGC  CCCCGCATCT  GCATCCGAAC
101  CTGCCGCCGC  TTCTCCGCG  CAGGGCGACA  CCTCTTCGAT  CGGCAGCAGC
151  ATGCAGCAGG  CAGCTATGC  GATGGCGGTG  GACATCGGAC  GCTCCCTGAA
201  CCAATGAGAG  CAGCGATGC  CGGAATCGA  TTGAAAGTC  TTTACCGAAG
251  CCAATGAGAG  AGTGTATGAC  GGCAAGAAJA  TCGAATATGAC  CGAAGAGCAG
301  GCTCAGGAAG  TCATGATGAA  ATTCTTTCAG  GAACAACAGG  CTAAGCCGT
351  AGAAAAACAC  AAGGGCGAG  CGAAGGCCAA  TAAAGAAAAA  GGCGAAGCCT
401  TTCTGAAGGA  AATGCGCGC  AAAGACGGCG  TGAAGACCAC  TGCTTCCGGT
451  CTGCATACCA  AATCACCACA  ACAGGGCGAA  GGCAACACAG  CGACAAAGA
501  CGACATCGTT  ACGTGGGAAT  ACGAAGCCGC  CCTGATTGAC  GGTACGGTAT
551  TCGACAGCAG  CAAAGCCAAC  GGGCGGCCGG  TCACCTTCCC  TTTGAGCCAA
601  GTGATTCGGG  GTTGACCGCA  AGGCGTACAG  CTTCTGAAGG  AAGCGGGCGA
651  AGCCACGTTC  TACATCCCGT  CCAACCTTGC  CTACCCGGA  CAGGGTGGCG
701  CGGCAAAAT  CGTCCGAAC  GCCACTTTGG  TATTTGATGT  GAACTGGTC
751  AAAATCGGCG  CACCCGAAAA  CGCGCCCGCC  AAGCAGCCGG  CTCAGTGC
801  CATCAAAAA  GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 27; ORF 576-1>:

```

m576-1.pep
1  MNTIFKISAL  TLSAALALSA  CGKKEAAPAS  ASEPARASSA  QGDTSSIGST
51  MQQASYAMGV  DGRSLQMKK  EQGAIEDLKV  FTEAMQAVYD  GKEIRMTTEQ
101  AQEVMMKFLQ  EQQAKAVEKH  KADAKANKKE  GEAFLEKNA  KDGVKTTASG
151  LQKTKTQGE  GKQFPRDDV  TVEYERGLID  GTVFDSSKAN  GGPVTFPLSQ
201  VIPGWTEGVQ  LLKGGGEATF  YIPSNLAYRE  QGAGDKIGPN  ATLVDVVKLV
251  KIGAPENAPA  KQPAQVDIKK  VH*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 28>:

```

g576-1.seq
1  ATGAACACCA  TTTTCAAAT  CAGCGCACTG  ACCCTTTCCG  CGGCTTTGGC
51  ACTTTCCGCC  TCGGCAAAA  AAGAAGCCGC  CCCCGCATCT  GCATCCGAAC
101  CTGCCGCCGC  TTCTCCGCG  CAGGGCGACA  CCTCTTCAAT  CGGCAGCAGC
151  ATGCAGCAGG  CAGCTATGC  AATGGCGGTG  GACATCGGAC  GCTCCCTGAA
201  ACAATGAGAG  GAACAGGCG  CGGAATCGA  TTGAAAGTC  TTTACCGAAG
251  CCAATGAGAG  CAGCGATGC  CGGAATCGA  TTGAAAGTC  TTTACCGAAG
301  GCGCAGGAAG  TGATGATGAA  ATTCTTTCAG  GAGCAGCAGG  CTAAGCCGT
351  AGAAAAACAC  AAGCGCGATG  CGAAGGCCAA  CAAAGAAAAA  GGCGAAGCCT
401  TCCTGAAGGA  AATGCGCGC  AAAGACGGCG  TGAAGACCAC  TGCTTCCGGT
451  CTGCAGTACA  AATCACCACA  ACAGGGTGAA  GGCAACACAG  CGACAAAGA
501  CGACATCGTT  ACGTGGGAAT  ACGAAGCCGC  CCTGATTGAC  GGTACCGTAT
551  TCGACAGCAG  CAAAGCCAAC  GGGCGGCCGG  CCACCTTCCC  TTTGAGCCAA
601  GTGATTCGGG  GTTGACCGCA  AGGCGTACGG  CTTCTGAAGG  AAGCGGGCGA
651  AGCCACGTTC  TACATCCCGT  CCAACTTTGC  CTACCCGGA  CAGGGTGGCG
701  GCGAAAAAT  CGTCCGAAC  GCCACTTTGG  TATTTGAGST  GAAACTGGTC
751  AAAATCGGCG  CACCCGAAAA  CGCGCCCGCC  AAGCAGCCGG  ATCAGTGC

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801 CATCAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 29; ORF 576-1.ng>:

g576-1.pep
 1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAAASAA OGDTS SIGST
 51 MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEG
 101 ACEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLEKNA KDVKTTTASQ
 151 LQYKITKQGE GKOPTKDDIV TVEYEGRLID GTVFDSKAN GGPATFFLSQ
 201 VIPGWTEGVR LLKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVDVKLV
 251 KIGAPENAPA KQPDQVDIKK VN*

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

	10	20	30	40	50	60
g576-1.pep	MNTIFKISAL	TLAALALSA	CGKKEAAPAS	ASEPAAASAA	OGDTS SIGST	MQQASYAMGV
m576-1	MNTIFKISAL	TLAALALSA	CGKKEAAPAS	ASEPAAASAA	OGDTS SIGST	MQQASYAMGV
	10	20	30	40	50	60
	70	80	90	100	110	120
g576-1.pep	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEG	QAEVMMKFLQ	EQQAKAVEKH
m576-1	DIGRSLKQMK	EQGAEIDLKV	FTDAMQAVYD	GKEIKMTEEG	QAEVMMKFLQ	EQQAKAVEKH
	70	80	90	100	110	120
	130	140	150	160	170	180
g576-1.pep	KADAKANKEK	GEAFLEKNA	KDKVKT TASGLQYKIT	KGEGKOPTKDDIV	TVEYEGRLID	
m576-1	KADAKANKEK	GEAFLEKNA	KDKVKT TASGLQYKIT	KGEGKOPTKDDIV	TVEYEGRLID	
	130	140	150	160	170	180
	190	200	210	220	230	240
g576-1.pep	GTVFDSKAN	GGPATFFLSQ	VI PGWTEGVR	LLKEGGEATF	YIPSNLAYRE	QGAGEKIGPN
m576-1	GTVFDSKAN	GGPATFFLSQ	VI PGWTEGVR	LLKEGGEATF	YIPSNLAYRE	QGAGEKIGPN
	190	200	210	220	230	240
	250	260	270			
g576-1.pep	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
m576-1	ATLVFDVKLV	KIGAPENAPA	KQPDQVDIKK	VN		
	250	260	270			

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq
 1 ATGAACACCA TTTTCAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
 51 ACTTTCCGCG TGCGGCACAAA AAGAAGCGCG CCCCGCATCT GCATCCGACG
 101 CTGCGCGCGG TTCTTCGCGG CAGGGCGACA CCTCTTCGAT CGGCAGCAGC
 151 ATGCAGCAGC CAGCTATGCG GATGGCGGTG GACATCGGAC GTCCCTCGAA
 201 GCAAATGAAG GAACAGGCGC CGGAATGCGA TTGAAAGTCG TTTACCGGAG
 251 CCATGCAGGC AGTGATGATG GGCAAGAGAA TCAAATGAC CGAAGAGCAG
 301 GCTCAGGAAG TCATGTGATA ATTCTTTCAG GAACAACAGG CTAAGCCGCT
 351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GCGCAGAGCTT
 401 TTCTGAAAGA AATGTCGCGC AAAGACGGCG TGAAGACCAC TGCTTCGCGT
 451 CTGCAATACA AATACACCAA ACAGGGCGAA GGCAACAGCG CGACCAAGA
 501 CGACATCGTT ACGGTGGAAT ACGAAGCGCG CCTGATTGAC GGTACGGTAT
 551 TCGACAGCAG CAAAGCCAAC GGGCGCCCGG TCACCTTCCC TTTGAGCCAA
 601 GTGATTCGCG GTTGGACGCA AGGGGTACAG CTTCGTAAG AAGGCGGCGA
 651 AGCCACGTTT TACATCCGCT CCAACCTTGC CTACCGCGAA CAGGCTGCGG
 701 GGCACAAAAA CGGCCCGAAC GCCACTTTGG TAITTTGATG TAACTGGTCT

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751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAGTCGA
801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep
1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAAASSA QGDTSSIGST
51 MQQASYAMGV DIGRSLKQMK EGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITKQGE GKQPTKDDIV TVEYGRLLD GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGGEATF YIPSNLAYRE QGAGDKIGPN ATLVDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

a576-1.pep	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV	10	20	30	40	50	60
m576-1	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV	10	20	30	40	50	60

a576-1.pep	DIGRSLKQMKEGAEIDLKVFTTEAMQAVYDQGEIKMTEEEQAQEVMMKFLQEQQAKAVEKH	70	80	90	100	110	120
m576-1	DIGRSLKQMKEGAEIDLKVFTTEAMQAVYDQGEIKMTEEEQAQEVMMKFLQEQQAKAVEKH	70	80	90	100	110	120

a576-1.pep	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQGEKGQPTKDDIVTVEYGRLLD	130	140	150	160	170	180
m576-1	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQGEKGQPTKDDIVTVEYGRLLD	130	140	150	160	170	180

a576-1.pep	GTVFDSSKANGGPVTFPLSOVILGWTEGVQLLKEGGGEATFYIPSNLAYREQGAGDKIGPN	190	200	210	220	230	240
m576-1	GTVFDSSKANGGPVTFPLSOVILGWTEGVQLLKEGGGEATFYIPSNLAYREQGAGDKIGPN	190	200	210	220	230	240

a576-1.pep	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN	250	260	270
m576-1	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN	250	260	270

919 and 919-2 gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq
1 ATGAAAAAAT ACCTATTCCG CGCGCCCTGT TACGGCATCG CGCGCCGCAAT
51 CCTCGCGCCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCGCTCAT CAACGCGCCG GACCGCGCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGCGCGCGG CGGCGCGCTG TATACCTTTG TACCGCACTT
201 GTCCCTTGCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
251 TCCGCTTCGG CTGCGCCCAAT TTGAAAACCC GCCAAGGCTG GCAGGATGTG
301 TGGCGCCCAAG CTTTCAAAC CCCTGCTCAT TCCTTTGAG CAAAACAGTT
351 TTTTGAACGC TATTTCACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG

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401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCGGATTTC GGTATTCCCG ACGATTTTAT
501 CTCGCTCCCC CTGCTCGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGCGGSCACA
601 CATACCGCCG ACCTTCGCG ATTCCGATC ACGCGGCGCA CAACAGCAAT
651 CAAGAGGAGC TTTCGAAGAA GCGCTTCCT CCGCTACCAC ACGGCGAATC
701 AAATCAACGG CGCGCGGCTT GACGCGAAG CCCGATACT CGGTACGCC
751 GAAGACCTGT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGCGGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGAT CGGCTATGCC GACAAAAAGC
851 AACATCCYTA CGTTTCCCAT GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAACCTCGAC AAACCTCCAT GCAGGCGATT AAGTCTTATA TGGCGAAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCGGGAAGC AGCAATGACG GCGCTGTGCG CGCACTGGGC
1051 ACGCGCGCTA TGGGGGAATA TGCCGCGGCA GTCGACGGC ACTACATTAC
1101 CTTGGGTGCG CCTTATTATT TGCGCACGCG CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACGC CTGATTATG GCGCAGGATA CGGCGAGCG GATTAAAGCG
1201 GCGGTGCGCG TGGATTATTT TTGGGATATC GCGCAGCAAG CCGGCGAACT
1251 TGCCGCGCAA CAGAAAACCA CGGGATATGT CTGCGACGTC CTACCCAAAG
1301 GTATGAAGCC GAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919.pep

```

1 MKKYLFRAL YGLAAAILAA CQSKSIOTFP QPDTSVINGP DRPVGIPDPA
51 GTTVGGGAV YTVVPHLSLP HNAQDFAKS LQSFRLGCAN LKMRGQMDV
101 CAQAFQTPVH SFOAKQFFER YFTVQVAGN GSLAGTVIGY YEPVLKGDOR
151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIOTGKN SGTIDNTGGT
201 HTADLSRFPI TARTTAIKGR FEGSRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPVYSI GRYMADKGYL
301 KLGQTSMQGI KSYMQRNPQR LAEVLGNPNS YIFFRELAGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVTAHPV TRKALNRLIM AODTGSARKG
401 AVRVDYFWGY GDEAGELAGK QKTGTVVWQL LPNGMKPEYR P*

```

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

m919-2.seq

```

1 ATGAAAAAAT ACCTATTCGG CGCGCGCCGT TACGGCATCG CGCGCGCAT
51 CCTCGCGCCC TGCCAAAGCA AGAGCATCCA AACCTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGCGCGG TCGGCATCCG CACCCCGCC
151 GGAACGACGG TCGCGCGCGG CGGCGCGCTG TATACCGTTG TACCGCACCT
201 GTCCCTGCC CACTGGCGGG CGCAGGATT CCACAAAAGC CTGCAATCCT
251 TCGGCTCGG CTGCGCCAAT TTGAAAACC GCCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTCAAC CCGCTCCAT TCCTTTGAG CAAAACAGTT
351 TTTTGAACGC TATTTACAGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAACCG TGCTGAGGG GACGACAGG
451 CGGAGCGCAC AGCCCGCTT CCGGATTAC GGTATTCCG AGGATTATT
501 CTCGCTCCCC CTGCTCGCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGCGGSCACA
601 CATACCGCCG ACCTCTCCG ATTCCCATC ACCGCGGCA CAACAGCAAT
651 CAAGGCGAGG TTGGAAGGAA GCGCTTCCT CCGCTACCAC ACGCGCAACC
701 AAATCAACGG CGCGCGGCTT GACGCGAAG CCCGATACT CGGTACGCC
751 GAAGACCTGT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGCGGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGAT CGGCTATGCC GACAAAAAGC
851 ACATTCCTCA CTTTTCATC GCGAGTATA TGGCGGATAA TGGCGGATAA
901 AAACCTCGAC AAACCTCCAT GCAGGCGATT AAGTCTTATA TGGCGAAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCGGGAAGC AGCAATGACG GCGCTGTGCG CGCACTGGGC
1051 ACGCGCGCTA TGGGGGAATA TGCCGCGGCA GTCGACGGC ACTACATTAC
1101 CTTGGGTGCG CCTTATTATT TGCGCACGCG CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACGC CTGATTATG GCGCAGGATA CGGCGAGCG GATTAAAGCG

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1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GCGCAGCAAG CCGCGCAACT
 1251 TGGCCGGCAA CAGAAACCA CGGGATATGT CTGGCAGCTC CTACCCAAACG
 1301 GTATGAAGCC CGAATACCG CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2.pep

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP OPDTSVINGP DRPVGIDPPA
 51 GTTVGGGGAV YTVVPHLSLP HWAQAQFPAK LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKOFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTAQAARFPYI GIPDDFISVP LPAGLRSGKA LVRIQTGKN SSTIDNAGST
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQNINGAL DGKAPILGYA
 251 EDPVLEFFMH IQSGRLKTP SGKYIRIGYA DKNHFPVSI GRYMADKGYL
 301 KLGQTSMQGI KSYMRFQPR LAEVLQNPFS YIFFRELAGE SNDGPVGAAG
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919.seq

1 ATGAAAAAAC ACCTGCTCCG CTCGCCCTG TACGGeatCG CCGCGGeatAT
 51 Cctcgcgcgc TGCCAAAGa gAGCATCCA AACCTTTCCG CAACCCGACa
 101 CATCCGTCAT CAACGGCCCG GACCGGCCCG CCGGCATCCC GCACCCCGCC
 151 GGAACGACGG TTGCGCGCGG GCGGCCCGCT TATACGTTG TGCGCCACCT
 201 GTCCATGCCC CACTGGCGGG CGCaggATT TTGCCAAAGC CTGCAATCTC
 251 TCGCGCTCGG TCGGCCAAT TTGAAAAAC GCCAAGGCTG GCAGATGTGT
 301 TCGCGCCAGG CCTTTCAAAC CCGCGTGCAT TCCTTTTCAG CAAAGcGgTT
 351 TTTTGAACGC TATTTCAAGC cgtGGCaggt tgcaggcaAC GGAAGcCTTG
 401 Caggtaagggt TACCGGCTAT TACGAACCGG TGCTGAAGGG GCAGCGCAGG
 451 CGGACGGAAC GGGCGCGCTT CCGGATTTAC GGTATTCCG ACGATTTTAT
 501 CTCCGTCGCG CTGCGTGCAG GTTTCGCGGG CGGAAAAAAC CTGTTCGCGA
 551 TCAGCGAGac ggGGAANAAC AGCGGCACGA TCGACAATGC CGGCGGCAGC
 601 CATACGCGCG ACCTCTCCCG ATTCCGCATC ACCCGCGCGA CAACGCGcaat
 651 caaaGGCAGG TTTGAaggAA CCGCGTCTCT CCTTACCAC ACAGCGCAACC
 701 AATcaacGG CGCGCGGCTT TTTCAATGCA AtccaggGCT CGGCGCCGCT
 751 GAagacCGc tCGaactCTT TTTCAATGCA AtccaggGCT CGGCGCCGCT
 801 GAAAAACCGc tCGGcaaat acatCCGAT cGgaTcGcG gaCAAAAGC
 851 AACAtcGgTa tGtttccatc ggACGctATA TCGCGCACAA AGCTACCTC
 901 AAGctcgggc agACCTCGAT GCAGGcctc aaagcTATA TCGCGCAAAA
 951 TCGCAACGCG CTGCGCGAAG TTTTGGGTCA AAACCCGAGC TATATCTTTT
 1001 TCCGCGAGCT TCGCGGAAGC GGCATTAAGG GCCCGTCGG CGCACTGGGC
 1051 ACGCCACTGA TGGGGGAATA GCGCGCGCA ATGACCGCG ACTACATTAC
 1101 CTGGGGCGCG CCTTATTG TGCGCACCGC CATCCGGTT ACCCGCAAAG
 1151 CCTCAACCG CCGTATTATG GCGCAGGATA CAGGCAAGCG GATCAAGGCT
 1201 GCGGTGCGCG TGGATTATTT TTGGGGTTAC GCGCAGCAAG CCGCGCAACT
 1251 TCGCGGCAA CAGAAACCA CGGGATATGT CTGCGAGCTC CTGCCAAGC
 1301 GCATGAAGCC CGAATACCG CCGTGA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919.pep

1 MKKHLLRSAL YGIAAAILAA CQSRSIQTFP OPDTSVINGP DRPAGIDPPA
 51 GTTVAGGGAV YTVVPHLSMP HWAQAQFPAK LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKRFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTERARFPYI GIPDDFISVP LPAGLRGGKN LVRIQTGKN SSTIDNAGST
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQNINGAL DGKAPILGYA
 251 EDPVLEFFMH IQSGRLKTP SGKYIRIGYA DKNHFPVSI GRYMADKGYL
 301 KLGQTSMQGI KSYMRFQPR LAEVLQNPFS YIFFRELAGE SNDGPVGAAG
 351 TPLMGEYAGA IDRYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

ORF 919 shows 95.9 % identity over a 441 aa overlap with a predicted ORF (ORF 919.ng)
from *N. gonorrhoeae*:

```

m919/g919
      10      20      30      40      50      60
m919.pep MKKYLFRRAALYGIAAAAILAACQSKSIQTFFPOPDTSVINGPDRPVGIPDPAGTTVGGGGAV
g919      - MKKHLRLSALYGIAAAAILAACQSRSIQTFFPOPDTSVINGPDRPAGIPDPAGTTVAGGGAV
      10      20      30      40      50      60

      70      80      90     100     110     120
m919.pep YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
g919      YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKRFFER
      70      80      90     100     110     120

      130     140     150     160     170     180
m919.pep YFTPWQVAGNGSLAGTVTGYEYFVLKGDGRRTAQARFPIYGIIPDDFISVPLPAGLRSKKA
g919      YFTPWQVAGNGSLAGTVTGYEYFVLKGDGRRTARERFPIYGIIPDDFISVPLPAGLRGKKN
      130     140     150     160     170     180

      190     200     210     220     230     240
m919.pep LVRI RQTGKNSGTIDNTGGHTADLSRFPITARTTAI KGRFEGRFLPYHTRNQINGGAL
g919      LVRI RQTGKNSGTIDNAGGHTADLSRFPITARTTAI KGRFEGRFLPYHTRNQINGGAL
      190     200     210     220     230     240

      250     260     270     280     290     300
m919.pep DGKAPILGYAEDPVLEFFMHIQSGSRLLKTPSGKYIRIGYADKNEHPVYSIGRYMADKGYL
g919      DGKAPILGYAEDPVLEFFMHIQSGSRLLKTPSGKYIRIGYADKNEHPVYSIGRYMADKGYL
      250     260     270     280     290     300

      310     320     330     340     350     360
m919.pep KLGQTSMQGIKSYMQRNPORLAEVLGQNPSYIFFRELAGSNDGVPVGLGATPLMGEYAGA
g919      KLGQTSMQGIKAYMRNPORLAEVLGQNPSYIFFRELAGSNEGVPVGLGATPLMGEYAGA
      310     320     330     340     350     360

      370     380     390     400     410     420
m919.pep VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELACK
g919      IDRH YITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELACK
      370     380     390     400     410     420

      430     440
m919.pep OKTTGYVWQLLPNGMKPEYRPX1
g919      OKTTGYVWQLLPNGMKPEYRPX
      430     440

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 38>:
a919.seq

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```

1  ATGAAAAAAT ACCTATTCCG CGCGGCCCTG TGCGGCATCG CGCGCCGCAT
51  CCTCCGCGCG TGCCAAAGCA AGAGCATCCA AACCTTCCG CAACCGGACA
101 CATCCGTCAAT CAACGGCCGG GACCGCGCGG TCGGCATCCC CGACCCCGCG
151 GGAACGACGG TCGCGGCGGG CGGGGCCGTT TATACCGTTG TGCCGCACTT
201 GTCCCTGCCG CACTGGGCGG CGCAGGATTT GCCTAAAGC CTGCATCCTT
251 TCGCGCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGCGTG CGAGGATGTG
301 TCGGCCAAG CCTTTCAAA ACCTGTCAT TCCGTTCAAG CAAAACAGT
351 TTTTGAACGC TATTTACGCG CGTGGCAGT TGCAGGCAAC GGAAGCCTTG
401 CGGTACGGT TACCGCTAT TACGAGCGGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AGCCCGCTT CCGGTTTAC GGTATTCGG ACGATTTTAT
501 CTCGTCGCC CTGCGTGGG GTTTGCGAG CGGAAAGCG CTGTGCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCCAAC TCGACAATC CGCGGCAACA
601 CATACCGCGC ACCTCTCCCA ATTCGCCATC ACTGCGGCGA CAACGGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCGGCTTCT CCCCTACCAC ACGGCGCAAC
701 AAATCAACGG CGGCGCGCTT GACGCGAAG CCCGATACT CGGTACCGCC
751 GAAGACCCCG TCGAACTTT TTTTATGCAC ATCCAAGCTT CGGGCGGTCT
801 GAAAAACCCG TCCGGCAAT ACATCCGCAT CGGCTATGCC GACAAAAAGC
851 AACATCCCTA CTTTCCATC GCACGCTATA TGGCGGACAA AGGCTACTCT
901 AAGCTCGGGC AGACCTCGAT GCACGCTATC AAAGCCTATA TGCAGCAAA
951 CCCGCAACGC CTCGCGAAG TTTTGGGCA AAACCCAGC TATATCTTTT
1001 TCCGAGAGCT TACCGGAAGC AGCAATGACG GCCCTGTGG CGCACTGGGG
1051 ACGCGCGCTGA TGGCGGAGTA CGCGCGGCGA GTGACCGCG ACTACATTAC
1101 CTTGGGCGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAG
1151 CCCTCAACGC CCGTATTATG GCGCAGGATA CCGGCAAGCG GATTAAAGCT
1201 GCGGTGCGCG TGGATTATT TTGGGGATAC GCGCAGAAAG CGCGGCACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT GTGGCAGCTT CTGCCCAACG
1301 GTATGAAGCC CGAATACCG CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

```

a919.pep
1  MKKYLFRAL CGIAAAILAA CQSKSIQTFF QPDTSVINGP DRPVGIPDPA
51  GTTVGGGAV YTVVPHLSL HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SVQAKQFFER YTFWQVAGN GSLAGTVTG YEVVLKGDDE
151 RTAQARFFIY GIPDDFISVP LFAGLRSGKA LVRIQTGKN SGTIDNTGTT
201 HTADLSQFFI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVI GRYMADKGYL
301 KLGQTSMQGI KAYMQNPQR LAEVLGNPNS YIFRELITGS SNDGPVGLG
351 TELMEYAGA VDRHYITLGA PLFVTAHPV TRKALNRLIN AQDTSGAIRG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

```

m919.pep      10      20      30      40      50      60
                MKKYLFRALYGLIAAAILAACQSKSIQTFQPDTSVINGPDRPVGIPDPAGTTVGGGAV
a919           10      20      30      40      50      60
                MKKYLFRALCGIAAAILAACQSKSIQTFQPDTSVINGPDRPVGIPDPAGTTVGGGAV

m919.pep      70      80      90      100     110     120
                YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHVSQAKQFFER
a919           70      80      90      100     110     120
                YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHVSQAKQFFER

m919.pep      130     140     150     160     170     180
                YTFPWQVAGNSLAGTVTGYEYFVLKGDDEDTAQAARFFIYGIPIPDFISVPLFAGLRSGKA
a919           130     140     150     160     170     180
                YTFPWQVAGNSLAGTVTGYEYFVLKGDDEDTAQAARFFIYGIPIPDFISVPLFAGLRSGKA

m919.pep      190     200     210     220     230     240
                LVRIQTGKNSGTIDNTGGTHTADLSRFITARTTAIKGRFEGSRFLPHTRNQINGGAL
a919           190     200     210     220     230     240
                LVRIQTGKNSGTIDNTGGTHTADLSRFITARTTAIKGRFEGSRFLPHTRNQINGGAL

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WO 00/66791

PCT/US00/05928

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```

a919      LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
           190      200      210      220      230      240
m919.pep  DGKAPILGYAEDPVELFFMHQGSRLKTPSGKYIRIGYADKNEHPVSIGRYMADKGYL
           250      260      270      280      290      300
a919      DGKAPILGYAEDPVELFFMHQGSRLKTPSGKYIRIGYADKNEHPVSIGRYMADKGYL
           250      260      270      280      290      300
m919.pep  KLGQTSMOGIKSYMQRNQPRLAEVLGQNPSYIFFRELAGSSNDGFPVGLGTPLMGEYAGA
           310      320      330      340      350      360
a919      KLGQTSMOGIKAYMQNQPRLAEVLGQNPSYIFFRELTSNDGFPVGLGTPLMGEYAGA
           310      320      330      340      350      360
m919.pep  VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFYFWGYGDEAGELAGK
           370      380      390      400      410      420
a919      VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFYFWGYGDEAGELAGK
           370      380      390      400      410      420
m919.pep  QKTTGYVWOLLPNGMKPEYRFX
           430      440
a919      QKTTGYVWOLLPNGMKPEYRFX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGCGATGCC GTACTGATAC GGTATGACGG CGGCAATGCG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCGCGCA GGTATGCGCG CCAATTGCTG
151 GATTTCGAGG ACACAGGCGC AGACGAACTG OACCGCAGCA GGATTTTGTC
201 GCAAGAACTC AGCCGCCTAT ATSCGCAAAC CGCCGCGGAA CTGCTGTGCA
251 GTCAAAACCT CGCACCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCGCGA ACACGGTTAC AGCATACAGC TTGCCGATTT
351 GCGCGCTCTG CGGXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
401 XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
451 XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
501 XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
551 XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
601 XXXXXXXCAGC TTCCTTACGA CAAAAACGGT GCAAAGTCGG CACAAGGCAG
651 CATATTGCCG CAATCTGCTG ACAGGCTGCT CGCCACCCCG TATTTCGCAC
701 AACGCCACCC TAAAGACACG GGGCGCGAAC TGTTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG GGAAGACCGA TACGACGTAT TCGGGACGCT
801 TTCCCGTTT ACCGCGCAAA CC GTTTCGCA GCGCGTCTCA CACGACGCGG
851 CAGATGCCCG TCAATGTGAC ATTTGCGACG CGCGGATCGC CAATCTGTTT
901 TTAATGGCGG ATTTGCGAGA ATGTTTCGCG ACACGCGTTT CCTGCAACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGATTGTT
1001 CGTGGTTGGC GGCCTGTGTT ATTAATCGCA TTCCCGGTAG TCCGCAACAA
1051 GCAACCGCGG CATCCAAACC GTGTATTCTG AnCGCGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 41; ORF 121>:

m121.pep

```

1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEHAFPT YFGRLLRRQLL
51  DLQQTGADEL HRSRLISQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGW

```

```

101 TVRHAPEHGY SIQLADLPLL AXXXXXXXXX XXXXXXXXXXXX XXXXXXXXXXXX
151 XXXXXXXXXXXX XXXXXXXXXXXX XXXXXXXXXXXX XXXXXXXXXXXX XXXXXXXXXXXX
201 xxQLPYDKNG AKSAQGNILF QLLDRLLAHP YFAQRHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICDGGRNPFV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAARAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGTGACGG
51 GCGCGATTGCC GTGCTGTGATC GATGTGACGG CGGCAAAATGG CTGGGCGCGGG
101 AAGGGCAGCG CTTTACCCCC TACCCTGACC GCTTGCSCCG GAAATGTCTG
151 GATTTCGAGG ACACAGGCAC AGACGAACCTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCGCGCTGT ACGCGCAAAAC GCGCGCGAA CTGCTGTGCA
251 GTCAAACCTC GCCTCCGTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCGCGA ACACGGTtac AGCATACAGC TTGCGGATT
351 GCGCGTGTCT GCGGAACCTGa cgcggatttt TACCGTCggc gaectccGCA
401 GCGCGGACCT TGCTGCCGCG GgacaAGGTG CGCGCTCGT CCCTGCTTTT
451 CACGAAGCCC TGTTCCCGAA TCACGCGAA ACACGCTGG TACTGAACAT
501 GCGCGGGATT GCCAACATCA GCGTACTGCC CCGCGGCGCA CGCGCTTGG
551 GCTTCGACAC AGGCGCGGGC AATATGCTGA TGGAcgctg gaecgagca
601 cacTGGcagc TGCTTACGA CAAAacggt gCAAGcgcg cacAGGCA
651 catatTGCcg cAACTGCTCG gcaggctGCT CGCCaccCG TATTTCAC
701 AACCCcacc aaAAAGCAGC GgCGGGAac TgtttgccT Aaattggctc
751 gaacacctAcc ttgacgcgcg cgaaaaccga tacgacgtat tgcggaact
801 ttcccgattc accgcgcaaa ccgTttggga gcgcgttca CACGCGACGG
851 CAGATGCCCG TCAATGTGAC ATTTCGCGCG CGGCGATCCG CAATCTGTT
901 TTAATGCGCG ATTTCGCGA ATGTTTCGCG ACACGCGCTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCTCAATG GTGCGAGCGG gcgcattg
1001 cgtggttggC GCGCTGTTGG ATTAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCGACCGGCG CATCCAAACC GTGTATTCTG GCGCGGGAT ATTATTATT
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMGADA VLVRMDGGKW LGAEGHAFTP YPDLRLRKLL
51 DLQGTGTDEI HRSRMLSQEL SRLYAQTAAE LLCSONLAPC DITALGCHGQ
101 TVRHAPEHGY SIQLADLPLL AELTRITFTV DFRSRDLAAG GGGAPLVPAF
151 HEALFRDRE TRVLNIGGI ANISVLPPGA PAFGFDITG NMLMDAWTQA
201 HQQLPYDKNG AKSAQGNILF QLLDRLLAHP YFQHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLSRF TAQTVWDAYS HAAADARQMY ICDGGRNPFV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGDADAVLVRMDGGKW	LGAEGHAFTPYPDLRLRQLLDLQDTGADEL				
g121	METQLYIGIMSGTSMGDADAVLVRMDGGKW	LGAEGHAFTPYPDLRLRQLLDLQDTGDEL				
	10	20	30	40	50	60
m121.pep	HRSRILSQELSRLYAQTAELLCSQNLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
g121	HRSRILSQELSRLYAQTAELLCSQNLAPCDITALGCHGQTVRHAPEHGYSIQLADLPLL					
	70	80	90	100	110	120
m121.pep						
g121						
	130	140	150	160	170	180

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```

m121.pep  AXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
g121      AELTRIPVGFDSRDLAGGQGAPLVPAFHEALFRDRETRVVLNIGGIANISVLPPGA
          130      140      150      160      170      180
          190      200      210      220      230      240
m121.pep  XXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLRLLAHYPFAQRHPKST
          :      :      :      :      :      :      :      :      :
g121      PAFGFDTPGFNMLMDAWTAQHOLPYDKNGAKAAQGNILPQLLRLLAHYPFSQPHPKST
          190      200      210      220      230      240
          250      260      270      280      290      300
m121.pep  GRELFALNWLETYLDGGENRYDVLRLTSRFTAQTVCDVSHAAADARQMYICDGGIRNPV
          :      :      :      :      :      :      :      :      :
g121      GRELFALNWLETYLDGGENRYDVLRLTSRFTAQTVWDVSHAAADARQMYICGGIRNPV
          250      260      270      280      290      300
          310      320      330      340      350      360
m121.pep  LMADLAECFGRVSLHSTADLNLDPOWVEAAFWLAACWINRIPGSPHKATGASKPCIL
          :      :      :      :      :      :      :      :      :
g121      LMADLAECFGRVSLHSTAEALNLDPOWVEAAFWLAACWINRIPGSPHKATGASKPCIL
          310      320      330      340      350      360

m121.pep  XAGYYYY
g121      GAGYYYY

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 44>:

```

a121.seq
1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGGATGCC GTACTGATAC GGATGACGCG CGGCAATGCG CTGGGCGCGG
101 AAGGGCAGCG CTTTACCCCG TACCCCGGCA GGTACGCGCG CAAATTGCTG
151 GATTTCGAGG ACACAGGCGC GGACGAAGTC CACCGCAGCA GGAATGTTGC
201 GCARGAACTC AGCCGCGCTG TACGCGCAAC CGCCGCGGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTGAGAC AGCGCGCGGA ACACAGTTCG AGCGTACAGC TTCCGCAATT
351 GCGCGTCTGG CGGGAACGGA CTCAGATTTT TACCGTGGCG GACTTCGCGA
401 GCGCGGACCT TCGGCGCGCG GGACAAGGCG CGCGCTCTGT CCGCGCTTTT
451 CACGAAGCCC TGTTCCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CCGCGGGATT GCCAACATCA GCGTACTCCG CCCCGCAGCA CCGCGCTTCG
551 GCTTCGACAC AGGACCGGCG AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGCGCAG TTCCTTACGA CAAAACCGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCGC CAACTGCTCG ACAGGCTGCT GCGCCACCGC TATTTCGCAC
701 AACCCGACCC TAAAGCAGC GGGCGCGAAC TGTTTGCCTT AAATTGCGTC
751 GAACCTACCC TTGACGCGCG CGAARACCGA TACGACGCTAT TCGGCGGCTT
801 TTCCCGATTC ACCGCGCGAA CCGTTTTCGA CGCCGTCTCA CACGACGGCG
851 CAGATGCCCG TCAATGTATC ATTTGCGCGC GCGGCATCCG CAATCCTGTT
901 TTAATGCGCG ATTGCGGAGA ATGTTTGGCG ACACGCGTTT CCCTGCACAG
951 CACCGCGCAA CTGAACCTCG ATCCGCAATG GGTAGAAGCG CGCGGTTGCT
1001 CATGGATGCG GCGCTGTGG GTCAACGCGA TTCCGCGTAG TCCGCACAAA
1051 GCAACCGCGC CATCCAACCG GTGTATTCTG GCGCGGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 45; ORF 121.a>:

```

a121.pep
1  METQLYIGIM SGTSMGADA VLIRMDGGKW LGAEGHAFPT YPGRLRRLKL
5  DLQDTGADEL HSRMSIQL SRYLAQTAKE LLCSQNLAPS DITALGCHQG
101 TVRHAPEHSY SVQLADLPLL AERTQIFTVG DFRSRLAAG GQGAPLVPAF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDANMQA
201 HWQLPYDKNG AKAAQGNILP QLRLRLLAHP YFAQHPKST GRELFALNWL
251 ETYLDGGENR YDVLRLTSRF TAQTVEDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPOWVEA AAFAMMAACW VNRIGPSPHK

```


351 ATGASKPCIL GAGYYY*

ml21/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

m121.pap		10	20	30	40	50	60
	METQLYTIGIMSGTSMGDGADAVLI	RMDGGKWLGAEGHAFTYP	PGRLRRQLLDLQDTGADEL				
a121	METQLYTIGIMSGTSMGDGADAVLI	RMDGGKWLGAEGHAFTYP	PGRLRRKLILDQDTGADEL				
	10	20	30	40	50	60	
m121.pap		70	80	90	100	110	120
	HRSRILSQELSRLYAQTAAELLCSQN	LPSDITALGCHG	GTVRHAEHGYISQLADLP				
a121	HRSRILSQELSRLYAQTAAELLCSQN	LPSDITALGCHG	GTVRHAEHGYISVQLADLP				
	70	80	90	100	110	120	
m121.pap		130	140	150	160	170	180
	XXXXXXXXXXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXXXXXXXXXXXXXXXXXX				
	:	:	:	:	:	:	:
a121	AERTQITVFGRSDRLAAGGQAPLV	PAFHEALFRDDR	TRAVLNGIGIANISVLPPDA				
	130	140	150	160	170	180	
m121.pap		190	200	210	220	230	240
	XXXXXXXXXXXXXXXXXXXXQLPY	RNKGAKSAQGNILL	POLLDRLLAHYPFAQRHPKST				
a121	PAFGFDTGFGNMLDAMWQAHQ	LPYDKNGAKAAGNILL	POLLDRLLAHYPFAQHPKST				
	190	200	210	220	230	240	
m121.pap		250	260	270	280	290	300
	GRELFAINWLETYLDGGENRYD	VLRTLSRFTAQTVCD	AVSHAAADAROMYICDGGIRNPV				
a121	GRELFAINWLETYLDGGENRYD	VLRTLSRFTAQTVF	DAVSHAAADAROMYICGGGIRNPV				
	250	260	270	280	290	300	
m121.pap		310	320	330	340	350	360
	LMADLAEFCFGRVLSHSTADN	LDPPQWEAAAFWLAACW	INRIPGSPHKATGASKPCIL				
a121	LMADLAEFCFGRVLSHSTAEIN	LDPPQWEAAAFWMAACW	VNIRIPGSPHKATGASKPCIL				
	310	320	330	340	350	360	
m121.pap		XAGYXXX					
a121		GAGYXXX					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEO ID 46>:

[illegible]

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```

801 TTCCCGTTTT ACCGCGCAAA CGSTTTGCGA CGCGGTCTCA CACGCGAGCGG
851 CAGATGCGCCG TCAATGTAC ATTGCGCGG CGCGCATCCG CAATCGTGTT
901 TTAATGGCGCG ATTGGGAGA ATGTTTCGGC ACACGCGTT CCTCGACAG
951 CACCGCGCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC CGCGNATTGT
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCOCACAAA
1051 GCAACGCGCG CATCCAAACC GTGTATTCTG ANCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

```

ml21-1.pep
1  METOLYIGIM SGTSMOGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRQLL
51  DLQDTGADEL HRSRLSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLP LL AERTRFTVVG DFRSRRLAAG GGGAFLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTPGG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRLTSRF TAQTVCDAMS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSHK
351 ATGASKPCIL XAGYYY*

ml21-1/g121      ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa
overlap

              10      20      30      40      50      60
ml21-1.pep    METOLYIGIMSGTSMOGADAVLIRMDGGKWLGAEGHAFTPYGRLLRQLLDLQDTGADEL
g121          METOLYIGIMSGTSMOGADAVLIRMDGGKWLGAEGHAFTPYDRLRRLKLDLQDTGTDEL
              10      20      30      40      50      60

              70      80      90      100     110     120
ml21-1.pep    HRSRLSQELSRLYAQTAAELLCSQNLAPSDITALGCHGQTVRHAPHEGYISQLADLP LL
g121          HRSRLSQELSRLYAQTAAELLCSQNLAPCDITALGCHGQTVRHAPHEGYISQLADLP LL
              70      80      90      100     110     120

              130     140     150     160     170     180
ml21-1.pep    AERTRFTVGDFRSRRLAAGGQGAFLVPFHEALFRDNRETRAVLNIGGANISVLPPDA
g121          AELTRFTVGDFRSRRLAAGGQGAFLVPFHEALFRDNRETRVVLNIGGANISVLPPGA
              130     140     150     160     170     180

              190     200     210     220     230     240
ml21-1.pep    PAFGFDTPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST
g121          PAFGFDTPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFSQPHPKST
              190     200     210     220     230     240

              250     260     270     280     290     300
ml21-1.pep    GRELFALNWLITYLDGGENRYDVLRLTSRFTAQTVCDAMSHAAADARQMYICGGGIRNPV
g121          GRELFALNWLITYLDGGENRYDVLRLTSRFTAQTVCDAMSHAAADARQMYICGGGIRNPV
              250     260     270     280     290     300

              310     320     330     340     350     360
ml21-1.pep    LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSHKATGASKPCIL
g121          LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSHKATGASKPCIL
              310     320     330     340     350     360

ml21-1.pep    XAGYYYX
g121          GAGYYYX

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

```

a121-1.seq
1   ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA CGATGGACGG
51  GGGCGATGCC GTACTGTATC GATGTGACGG CGGCARATGG CTGGGGCGGG
101 AAGGGCAGCG CTTTACCCCC TACCOCGGCA GGTTAGCCCG CAAATTGCTG
151 GATTTCAGCG ACACAGGGCG GACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAAGCT AGCCGCGCTG AGCGCAAAAC CGCCGCGGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCGCTCC GACATTACCG CCCTGGGCTG CCACGGGCAA
301 ACCGTCAGAC AGCGCGCGGA ACACAGTTAC AGCGTACAGC TTGCGGATTT
351 GCGGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCGGCA
401 GCGGCGACTC TCGCGCGCGC GCACAAGGCG CGCCGCTGCT CCGCGCTTTT
451 CACGAAGCCC TGTTCGGCGA GCACAAGGAA ACACGCGCGG TACTGACAT
501 CGCGGGGATT GCCAACATCA CGCTACTCCC CCGCAGCA CAACGCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGT GATGCAGGCA
601 CACTGGCAGC TTCCTTAGCA CAAAACCGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT GCACCACCGG TATTTCGCAC
701 AACCCACCCC TAAAAGCAGC GGGCGCGAAC TGTTCGCCTT AAATTCGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACGGA TACGACGTAT TCGGACGCTT
801 TTCCCGATTG ACOCGSCAAA CGSTTTTCGA CGCGCTCTCA CACGACGCGG
851 CAGATGTCGG TCAAAATGAC ATTTCGGCGC GGGCATATCG CAATCTCTTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCTGACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCCGGCTCG
1001 CATGGATGCG GCGCTGTGG GTCAACCGCA TTCCGCGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG GCGCGGGGAT ATTTATTATT
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

```

a121-1.pep
1   METQLYIGIM SGTSMGADA VLIRMDGGKW LGAEGHAFTP YPGRRLRRKLL
51  DLQDTGADEL HRSRLSQEL SRLYAQTAAE LLCQNLAAPS DITALGCHGQ
101 TVRHAPFHSY SVQLADLP LL AERTQITFVG DFRSRDLAAG GGCAFLVPF
151 HEALFRDRE TRAVLNIGGI ANISVLPPDA PRGFDTPGPG NMLDAWQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRLTSLR FTAQTVFDAVS HAAADARQMY ICGGGIRNFW
301 LMDLAECFG TRVSLHSTAE LNLDPQWVEA AAFAWMAACW VNRIGPSPHK
351 ATGASKPCIL GAGYYY*

```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGADAVLIRMDGGKWLGAEGHAFTPYPGRRLRQLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMGADAVLIRMDGGKWLGAEGHAFTPYPGRRLRKLDDLQDTGADEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRLSQELSRLYAQTAAELLCQNLAAPSDITALGCHGQTVRHAEPHSGYSIQLADLP LL					
a121-1	HRSRLSQELSRLYAQTAAELLCQNLAAPSDITALGCHGQTVRHAEPHSGYSVQLADLP LL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTQITFVGDFRSRDLAAGGQGAFLVPFPAHEALFRDRETRAVLNIGGTANISVLPPDA					
a121-1	AERTQITFVGDFRSRDLAAGGQGAFLVPFPAHEALFRDRETRAVLNIGGTANISVLPPDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGNMLMDAWQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGNMLMDAWQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240

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	250	260	270	280	290	300
m121-1.pep	GRELFLALNWLET	YLDGGENRYD	VLRTLSRFTA	QTVCDVSHAAADAR	QMYICGGGIRNPV	
a121-1	GRELFLALNWLET	YLDGGENRYD	VLRTLSRFTA	QTVCDVSHAAADAR	QMYICGGGIRNPV	
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGR	TVSLHSTAD	NLDQWVEAA	XFawLAACW	INRIPGSPH	KATGASKPCIL
a121	LMADLAECFGR	TVSLHSTAD	NLDQWVEAA	XFawLAACW	INRIPGSPH	KATGASKPCIL
	310	320	330	340	350	360
m121-1.pep	XAGYYYY					
a121	GAGYYYY					

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

```

m128.seq (partial)
1  ATGACTGACA AGCCTACTGCT CCATTGCGGC GAAGAACCCC GTTTTGATCA
51  AATCAAAACC GAAGACATCA AACCGCCCTT GCATAACGCC ATCGCCGAAG
101 CGCGCGAACA AATCGCGGCC ATCAAAGCCC AAACGCACAC CGCTGGGCA
151 AACACTGTGC AACCCCTGAC CGGCATCACC GAACCGCTCG GCAGGATTGT
201 GGGCGTGGTG TCGCACTCA ACTGCGTCGC GCACAGGCC GAACGCGCG
251 CGGTCTATAA CGAACTGATG CCGGAAATCA CCGTCTCTT CACCGAAAT
301 GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTCGAC ACCCTCTCCC CGGCACAAAA AACCAAACTC AACCAC
1  TACGCCAGCG AAAAAGTGGC CGAAGCCAAA TACGCGTTCA GCGAAACCGA
51  WGTCAAAAAA TAYTCCCYG TCGCGAAGGT ATTAAACGGA CTGTTCGCC
101 AATTCAAAAA ACTTACGGC ATCGGATTGA CCGAAAAAAC GTTCCCGCT
151 TGGCACAAG ACGTGCGCTA TTKTGAATTG CAACAAAACG GCGAACCAT
201 AGCGCGCGTT TATATGGATT TGTACGACG CGAAGGCCAA CGCGCGCGCG
251 CTGTGATGAA CGACTACAAA GCGCGCGCCG GTTTTTCAGA CGGCACGCTG
301 CAATGCCCCA CGGCTACTCT CGTCTGCAAC TTCGCGCCAC CGGTGCGCG
351 CAGGGAAGCC CGCYTAGGCC ACGACGAAAT CCTCATCTCT TTCCACGAAA
401 CCGGACAGCG GCTGCACCAC CTGCTTACCC AAGTGGACGA ACTGGCGGTA
451 TCGCGCATCA ACGCGGTAKA ATGGGACGCG GTCGAACTCG CCAGCCAGTT
501 TATGAAAAAT TTGCTTTGGG AATACAAATG CTTGGCACA mTGTGAGCC
551 ACGAAGAAGC CGGCGTTCCC YTGCGGAAG AACTCTTGA CAAATGCTC
601 CGCGCAAAAA ACTTCCAAGs CGGCTGTTT YtGTGCGG CAATGGATTG
651 CGCCCTCTTT GATATGATGA TTTACAGCGA AGACGACGAA GGCGCTCTGA
701 AAAACTGGCA ACAGGTTTGA GACAGCGTGC GCAAAAAAGT CGCGCTCATC
751 CAGCGCGCCG AATCAACCG CTTCGCGCTG AGCTTCGCGC ACATCTTCG
801 AGCGCGCTAT TCGCGAGCTT ATTAACAGTA CGCGTGGCGG GAATATTGA
851 CGCGCGACCG ATACGCGCCC TTGGAAGAAA GCGAGCATGT CCGCGCACCA
901 GCGAAACCGT TTTGGCAGA AATCTCGCC GTCGGGGAT CGCGCAGCG
951 nGCAGATCC TTCAAGCCT TCGCGCGCG CGAACCGAGC ATGACGCGAC
1001 TCTTGGCCA CAGCGGTTT GACAACGGGG TCTGA

```

This corresponds to the amino acid sequence <SEQ ID 51; ORF 128>:

```

m128.pep (partial)
1  MIDNALHLGL EEPFDDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGWA
51  NTVPELTGIT ERVGRWGVV SHLNCVADPT ELRAVYNELM PEITVFPTBI
101 GQDIELYNRF KTIKNSPEFD TLPQAKTKL NH
//
1  YASEKLREAK YAFSETXVKK YFPVGKVLNG LFAQXKKLYG IGFTETKVPV

```

```

51  WHKDVRVYXEL QQNGEXIGGV YMDLYAREGK RGGAWMNDYK GRRRFSDBGTL
101  QLPTAYLVNLC FAPPVUGGREA RLSHDEILIL FHETGHGLHH LLTQVDELVG
151  SGINGVXWDA VELPSOFMEN FVWEYNVLAQ XSAHEETGVP LPKELXDKXL
201  AAKNFQXGFM VXRQXEFALP DMIIYSEDD ERLKNQVQL DSVRKKVAIV
251  QPPEYNRFAL SFGHI FAGGY SAAXSYAWA EVLSADAYAA FEESDDVAAT
301  GKRFPQRIILA VGXSRSGAES FKAFRGREPS IDALLRHSGF DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

g128.seq

```

1  atgattgaca acgCactgct ccacttgggc gaagaaccCC GTTTTaatca
51  aatccaaacc gaagACatca AACCcCGCGT CCAAAcCGCC ATCGCCGAAG
101  CGCGCGGACA AATCGCGGCC GTCAAAGCGC AAACGCACAC CGCGTGGCG
151  AACACCGTCG AGCGTCTGAC CGGCATCACC GAACCGTCG CGAGGATTG
201  GGGCGTCGTG TCCCATCTCA ACTCGTCGTG CGACACGCCG GAATCGCGCG
251  CGGTCTATAA CGAACTGATG CCTGAAATCA CGGTCTCTT CACCGAAATC
301  GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351  CGAATTGTCG ACGCTTTCCC CGGCACAAAA AACCAAGCTC GATCAGACC
401  TGCGCGATTG CATTATTGAGC GCGCGGGAAC TGCGCGCCGA ACGGACGGCA
451  GACTGCGCAA AACTGCAAC CGAAGCGCGT CAACCTTCGG CCAAACTTCTC
501  CCAAAACGTC CTAGAGCGCA CGACCGCTTC CGCATCTAC TTGACGATG
551  CGCACCGCGT TGCGCGCATT CCGAAGACG CGCTCGCAT GTTTCCGCC
601  GCGCGCGCAA GCGAAGGCAA AACAGGTTAC AAAATCGCT TCGCATTTCC
651  GCATACCTTT GCGGTTATCC AATACGCGCG CAACCGCGAA CTGCGCGAAC
701  AAATCTACCG CGCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751  AAATTCGACA ACACCGGCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801  AACCGccaaa cTGCTCGGCT TTAATAATTA CGCGGAATTG TCGTGCGCAA
851  CCAAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCT CTACGACCTC
901  GCGCGCGCGC CCAAAACCTA GCGCGAAAAA GACCTCGCG AGTCAAAGC
951  CTTCGCGCGC GAACACCTCG GTCTCGCGGA CCGCGAGCGG TGGGACTTGA
1001 GCTACGCGCG CGAAAACTG CGCGAAGCCA AATACGCAT CAGCGAAATC
1051 GAAGTCAAAA AATACTTCCC CGTCGSCAAA GTTCTGGCAG GCCTGTTCGC
1101 CCAATCAAAA AAATCTACG GCATCGGATT CGCGAAAAAA ACCGTTCGCG
1151 TCTGGCACAA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGCGCGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AAGCGCGCGG
1251 CGCGTGATG AACGACTaca AAGCGCGCGC CGCGTTTGGC GAGCGcaGCG
1301 TGCGATGCGC CACCGCTTAC CTGCTGCGA ACTTCGCCCC GCGCGTGGC
1351 GCGAAGAGAG CGCGTTTAA GCTCGACGCA ATCTGACCC TCCTTCAGCA
1401 AacCGGCGAC GACCTGCACC ACCTGCTTAC CCAAGTGAGC GACTCGCGC
1451 TGTCGCGCAT CAacggcgtA GAATGGGAGC CGGTGCAAT GCCAGCGAG
1501 TTTATGGAAA ACTTCGTTTG GGAATACAAT GTATTGGCAC AAATGTCCGC
1551 CCACGAGAGAA ACGCGGAGC CCCTGCCGAA AGAACTCTTC GACAAATGCG
1601 TGcGCGCCAA AAATCTCCAG CGCGGTATGT TCCTCGTCCG GCAAAATGAG
1651 TTCGCCCTCT TCGATATGAT GATTTACAGT GAAAGCGAGC AATGCCGTCT
1701 GAAAAACTGG CAGCAGGTTT TAGACAGCGT GCGCAAGAA GTcGCGCTG
1751 TCCAACCGCC GGAATACACG CGCTTCGCCA ACAGCTTCGG CcacatctTC
1801 GCGggCGGCT ATTCGCGAGG CTATTACAGC TACGATGGG CCGAGTCTct
1851 cAGCACCGAT CGCTACGCGC CTTTGAAGA AAGcGACGac ctCGCGCAGC
1901 CAGGCAACCG CTCTGSCAA GAAATccttg cgtcgcgcg ctCCGCGAGC
1951 gcgCGGAAT CTTTCAAGC CTTCGCGGA CGCGAACGGA CGATAGACCG
2001 ACTGCTGCGC CaaagcggT TCGACACGC gGcttga

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

g128.pep

```

1  MIDNALLHLG EEPFNQIQT EDIKPAVQTA IAEARGQIAA VKAQTHGTWA
51  NTVRLTGIT ERVGRIGWVV SHLNSVDPT ELRAVYNELM PEITVFFTEI
101  GQDIELYNRF KTIKNSPEFA TLPSPAQTKL DHDLRDFVLS GAELPPERQA
151  ELAKLQTEGA QLSAKFSQNV LDATDAFGTY FDDAAPLAGI PEDALAMFAA
201  AAQSEKGTGY NIGLQIPHYL AVIQYAGNR LREQIYRAYV TRASELSNDG

```

550 560 570 580 590 600

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```

g128. pep      LVRQMEFALFDMMIYSEDECRLLKNWQVLDVSRKEVAVIQPEYNRFANSFGHIFAGGY
                |||
m128           XVRQXEFALFDMMIYSEDEGRLLKNWQVLDVSRKKVAVIQPEYNRFALSFGHIFAGGY
                |||
                220      230      240      250      260      270

g128. pep      610      620      630      640      650      660
                SAGYSYSAWAEVLSLTDAYAAPESDDVAATGKRFWQEILAVGGSRAAESPKAFRGREPS
                |||
m128           SAAXYSYSAWAEVLSADAYAAPESDDVAATGKRFWQEILAVGXSRGAESPKAFRGREPS
                |||
                280      290      300      310      320      330

g128. pep      670      679
                IDALLRQSGFDNAAX
                |||
m128           IDALLRHSGFDAVX
                |||
                340

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

```

a128.seq
1   ATGACTGACA  ACGCACTGCT  CCATTGGGCG  GAAGAACCCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCCTGCCCT  GCAAAACGCC  ATTGCGGAAG
101 CGCGCGAACA  AATCGCGGCC  ATCAAGAGCCC  AAGCGACAC  CGGCTGGGCA
151 AACACTGTGC  AACCCTGTAC  CGGCATCACC  GAACGGGTGC  GCAGGATTTC
201 GGGCGTGTGT  TCGCACTCTA  ACTCGCTCAC  CGACAGCCGC  GAAGTGGCGG
251 CCGCTACAAA  TGAATTAATG  CCGGAAATTA  CGCTCTCTTT  CACGAAATTC
301 GGACAAGACA  TCGAGCTGTA  CAACCGCTTC  AAAACATCA  AAACCTCCCC
351 CGAGTTCGAC  ACCCTCTCCC  ACGCGCAAAA  AACCAAACTC  AACCACGATC
401 TCGCGGATTT  CGTCTCAGC  GGGCGGGAAC  TGGCGGCCGA  ACAGCAGGCA
451 GAATTGGCAA  AACTGCAAAC  CGAAGGCGCG  CAATTTCCG  CCAATTTCTC
501 CCAAAACGTC  CTAGACGCGA  CCGACGCGTT  CGGCATTAC  TTTGACGATG
551 CGGCACCGCT  TGGCGGCATT  CCGGAAGAGC  CGCTGGCAT  GTTTGCGGCT
601 GCGCGGCAAA  GCGAAGGCAG  AAGCAGGCTAC  AAAATCGGTT  TGCAGATTCC
651 GCATCTACCT  GCGCTATCC  AATACGCGCA  CAACCGCAAA  CTGCGCGAAC
701 AAATCTACCG  CGCTACGTT  ACGCGGCCCA  CGAGCTTTTC  AGACGACGCG
751 AAATTCGACA  ACACCGCCAA  CATCGACCGC  ACGCTCGAAA  ACGCCTTGCA
801 AACCGGCCAA  CTGCTCGGCT  TCAAAACATA  CGCGGAATTG  TCGCTGGCAA
851 CCAAAATGGC  GGACACCCCC  GAACAAGTTT  TAACTTCTCT  GCACGACCTC
901 GCCCGCGCG  CCAAAACCTA  CGCGCAAAA  GACCTCGCG  AAGTCAAGCG
951 CTTGCGCCGC  GAAAGCCTCG  GCCTCGCCGA  TTTGCAACCG  TGGGACTTTG
1001 GCTACGCGCG  CGAAAAACTG  CGCGAAGCCA  AATACGCATT  CAGCGAAACC
1051 GAAGTCGAAA  AATACTTCCC  CGTCGGCMAA  GTATTAAACG  GACTGTTTCG
1101 CCAATACAA  AACTGCTAGC  GATCGGATTC  TACGCAAJAA  ACCGTCCCGC
1151 TCTGTGACAA  AGACGTGGCG  TATTTGGAAT  TGCACAAAAA  CGGCGAACC
1201 ATAGCGCGCG  TTTATATGGA  TTTGTACGCA  CGCGAAGGCA  AACGCGGCGG
1251 CGGCTGGATG  AAGCACTACA  AAGGCGCGCG  CGGTTTTTCA  GACGGCACGC
1301 TGCAACTGCC  CACCGCTTAC  CTCGTCTGCA  ACTTCACCCC  GCGCGTCGCG
1351 GGCAAGAAG  CCGCGTTGAG  CATGACGAA  ATCCTACCCC  TCTTCCACGA
1401 AACCGGACAC  GGCTTGCACC  ACCTGCTTAC  CCAAGTCGAC  GAAGTGGGCG
1451 TATCCGGCAT  CAACGGCGTA  GAATGGGAGC  CAGTCGAATC  GCGCAGTTCG
1501 TTTATGMAA  ATTTGCTTTG  GGAATACAAT  GTCTTGGCGC  AAATGTCCGC
1551 CCACGAGAA  ACGGCGTTC  CCTCGCGAA  AGACTCTTC  GACAAATGCG
1601 TCGCGGCGAA  AACTTCCAA  CGCGGAATCT  TCCTCGTCCC  CGAAATGAGG
1651 TTCGCCCTCT  TTGATATGAT  GATTTCACGC  GAAGACGACG  AAGCGCGTCT
1701 GAAAAACTGG  CAACAGGTTT  TAGACAGGCT  GCGCAAGAAA  GTGCGCGTCG
1751 TCCGACCGCC  CGAATACAAC  CGCTTCGCCA  ACAGCTTCGG  CCACATCTTC
1801 GCAGGCGGCT  ATTCGCGAGG  CTAATTACAG  TACGCTGGG  CGGAAGTATT
1851 GAGCGCGGAC  GCATACGCC  CCTTTGAAGA  AAGCGACGAT  GTGCGCGCCA
1901 CAGGCAAAAC  CTTTGGCGAG  GAAATCTCTG  CGGTCGGCGG  ATCGCGCAGC
1951 CGGCGCAAGT  CTTTCAAGCG  CTTCCGCGGA  CGCGAACCAG  GCATGAGCGT
2001 ACTCTTGGCG  CACAGCGGCT  TCGACAACAC  GGCTTGA

```

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This corresponds to the amino acid sequence <SEQ ID 55; ORF 128.a>:

```

a128.pep
1  MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQTHTGWA
51  NTVEPLTGIT ERVGRWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEKGTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFONTANIDR TLENALQPAK LLGFQVYAEI SLATKMDTP EQVNLFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLQF WDLGYAGEKL REAKYAFSE
351 EVKKYFPVKG VLNGLFAQIK KLYGIGFTEK TVPVVHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTLLQLPTAY LVCNFTFPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSINGVW EWDDELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAANKFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRKLNW QOVLDSVRKE VAVVRPPEYN RFANSFGHIF
601 AGGYSAGYYS YAAEVLSDAD AYAAFEESDD VAATGKRFRWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFNA**

```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```

m128.pep      10      20      30      40      50      60
                MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAKAQTHTGWANTVEPLTGIT
a128          10      20      30      40      50      60
                MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAKAQTHTGWANTVEPLTGIT

m128.pep      70      80      90      100     110     120
                ERVGRWGVVSHLNCVADTELRVAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
a128          70      80      90      100     110     120
                ERVGRWGVVSHLNSVTDTPELRVAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD

m128.pep      130
                TLSPAKTKLNH-----
a128          130      140      150      160      170      180
                TLSHAQKTKLNHLDRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY

m128.pep      -----
a128          190      200      210      220      230      240
                FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV

m128.pep      -----
a128          250      260      270      280      290      300
                TRASELSDDGKFONTANIDRTLENALQPAKLLGFQVYAEISLATKMDTPQOVLNLFHDL

m128.pep      140      150
                -----YASEKLREAKYAFSETXVKKYFPVVGX
a128          310      320      330      340      350      360
                ARRAKPYAEKDLAEVKAFARESLGLADLQFWDLGYAGEKLREAKYAFSETEVKKYFPVVGK

m128.pep      160      170      180      190      200      210
                VLNGLFAQIKKLYGIGFTEKTVPVVHKDVRYEELQONGETIGGVYMDLYAREGKRGGAWM
a128          370      380      390      400      410      420
                VLNGLFAQIKKLYGIGFTEKTVPVVHKDVRYEELQONGETIGGVYMDLYAREGKRGGAWM

m128.pep      220      230      240      250      260      270
                NDYKGRRRFSDGTLLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD

```


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```

|||||
a128 NDYKGRRRFSDGTLQLPTALVNCNFTFPVVGKEARLSHDEILTLFHETGHGLHLLTQVD
      430      440      450      460      470      480

      280      290      300      310      320      330
m128.ppe ELGVSGINGVXWDVADELPSQFMENFVWEYNVLAQMSAHEETGVPLPEKELMDXLLAAKNFQ
      |||||
a128 ELGVSGINGVEWDVADELPSQFMENFVWEYNVLAQMSAHEETGVPLPEKELMDXLLAAKNFQ
      490      500      510      520      530      540

      340      350      360      370      380      390
m128.ppe XGMFXVRQKEFALFDMMIYSEDDDEGRLEKNQVQLDSVRKKVAVIOPPEYNRFALSGFHIF
      |||||
a128 XGMFLVRQKEFALFDMMIYSEDDDEGRLEKNQVQLDSVRKEVAVVRPEYNRFALSGFHIF
      550      560      570      580      590      600

      400      410      420      430      440      450
m128.ppe AGGYSAAXSAYAAEVLADAYAAFEESDDVAATGKRFWQEIILAVGXSRSGAESFKAFRG
      |||||
a128 AGGYSAGYSYAAEVLADAYAAFEESDDVAATGKRFWQEIILAVGGSRAESFKAFRG
      610      620      630      640      650      660

      460      470
m128.ppe REPSIDALLRHSGFDAVAX
      |||||
a128 REPSIDALLRHSGFDAAX
      670

```

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 56>:

```

m128-1.seq
1 ATGACTGACA ACGCACTGCT CCATTGCGGC GAAGAACCCC GTTTTGATCA
51 AATCAAAACC GAAGACATCA AACC CGCCCT GCAAACCGCC ATCGCGGAAG
101 CGCGCGAACA AATCGCGGCC ATCAAGGCCC AAACGCACAC CGCGTGGCA
151 AACACTGTGC AACCCTGAC CGGCATCACC GAACGCGTC GCAGGATTG
201 GGGCGTGGTG TCGCACTCA ACTCGTGGC GCACACGCCC GAATCGCGCG
251 CGTCTATATA CGAACTGATG CCGGAATCA CCGTCTTCT CACCGAAATC
301 GGACAGACA TCGAGCTGTA CAACCGCTTC AAAACATCA AAAATTCGCC
351 CGAATTGAC ACCCTCTCCC CGGCACAAAA AACCACACTC AACCAGATC
401 TGCGCGATT CTCTCTCGC CGCGCGGAAC TGCGCGCGCA ACAGCAGGCA
451 GAACCTGCAA AACTCGAAAC CGAAGCGCGC CAACCTTCCG CCAAAATTC
501 CCAAAACGTC CTAGACGCGA CCGACCGGTT CGCATTTAC TTTGACATG
551 CCGCACCGCT TGCGCGGCATT CCGGAAGACG CGCTCGCCAT GTTTGCGGCC
601 GCGCGCGAUA GCGAAAGCAA AACAGGCTAC AAAATCGGCT TGCAGATTCC
651 ACACACTCTC GCCGTCTATC AATACGCCGA CAACCGCGAA CTGCGGGAAC
701 AACTCTACCG CGCTCTAGTT ACCCGCGCCA GCGAACTTTC AGACGACGGC
751 AACTTCGACA ACACCGCGAA CATCGACCGC ACGCTCGCAA ACGCGTGGCA
801 AACCGGCCAA CTGCTCGGCT TCARAAACTA CGCCGAATTG TCCTGCGCAA
851 CCAAAATGCG GCACACGCCC GAACAAGTTT TAAACTTCTT GCACGACCTC
901 GCCCGCGCGC CCAAAACCTA CGCCGAAAAA GACTCGGCC AACTCAAGC
951 CTTCGCGCGC GAAAGCGTGA ACCTCGCGCA TTGCAACCG TGCGACTTGG
1001 GCTACGCCAG CGAAAAACTG CGCGAAGCCA AATACCGGTT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAAA GTATTAAAGC GACTGTTCGC
1101 CCAAAATCAA AAATCTTACG GCATCGGATT TACCGAAAAA ACCGTCCCGC
1151 TCTGGGCACA AGACGTGGCG TATTTTGAA TGCACAAAAA CGCGGAACCC
1201 ATAGGCGCGC TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGCGCG
1251 CGCGTGGATG AACGACTACA AAGCGCGCGC CGGTTTTTCA GACGCGACCG
1301 TGCACTGCC CACGCGCTAC CTGCTGTGCA ACTTCGCCCC ACCCGTCGGC
1351 GCGACGGAAG CCGCGCTGAG CCACGACGAA ATCTCATC TCTTCAGA
1401 AACCGGACAC GGGCTGCACC ACCTGCTTAC CCAAGTGAC GAATCGGGC

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1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CGGTGGAAC TCCAGCCAG
1501 TTTATGGAAA ATTTCTGTTG GGAATACAAT GTCTTGGCAC AAATGTGAGC
1551 CCACGAAGAA ACCGGCGTTC CCTGCGCGAA AGAAGCTCTC GACAAAATGC
1601 TCGCCGCGAA AACTTCCAA CGCGGCATGT TCTCGTCCG GCAATGGAG
1651 TTCCGCCCTCT TTGATATGAT GATTTACAGC GAAGACGACG AAGCGCGTCT
1701 GAAAACTCGG CACACAGGTT TAGACAGCGT GCGCAAAAAA GTCCGCGTCA
1751 TCCAGCGGCC CGATACAAC CGCTTGCCT TGAGCTTCGG CCACATCTTC
1801 CGAGCGCGCT ATTCGCGAG CTATTACAGC TACGCTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCGC CTTTGAAGA AAGCGACGAT GTCCGCGCCA
1901 CAGGCCAAACG CTTTGGCAG GAAATCCTCG CCGTCGCGCG ATCCGCGACG
1951 GCGGCAGAAAT CTTTCAAAGC CTTCCGCGCG CCGCAACCGA GCATAGACGC
2001 ACTCTTGGCG CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1.pep.

```

1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGWGA
51 NTVEPLTGIT ERVGRINGVV SHLNSVADTP ELRAVYNELM PEITVFFTEI
101 GQDIELNRF KTIKNSFEED TLSPAQTKL NHDLRDFVLS GAELPFEQQA
151 ELAKIQTGA QLSAKFSQNV LDDAFAGYI FDDAAPLAGI PEDALAMFA
201 AAGSESKTGY KIGLIPIHYL AVIQYADNRE LREQTYRNV TRASELSDG
251 KFDNTANIDR TLANALQTA LGLFKNYAEL SLATKMAOTP EQVNLFDHL
301 ARRAKPYAEK DLAEVKAFAR ESLNLADLPQ WDLGYASEKL REAKYAFSET
351 EVKKYFFVGK VINGLFAQIK KLYGIGFTEK TVPVHKKDVR YFELQONQET
401 IGGVYMDLYA REGKRGGAWM NDYKRRRFS DGTLLQPTAY LVCHFAAPPVQ
451 GREARLSHDE TILIFHETGH GLHLLTQVD ELGVSGINGV EWDVAVELPSG
501 FMEFWWEYN VLAQMSAHEE TGVPLPKELF DKMLAKNFQ RGMFLVRQME
551 FALFWMYKQ FALFWMYKQ QVLDVSRKK VAVIOPPEYN RFALSFGHIF
601 AGYSAGYYS YAAAEVLSAD AYAAFEESDD VAATGKRFQW EILAVGGSR
651 AAESFAKFRG RPPSIDALLR HSGFDNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1 ATGATTGACA ACGCACTGCT CCACTTGGGC GAAGAACCCC GTTTTAATCA
51 AATCAAAACC GAAGACATCA AACC CGCGCT CCAAACCGCC ATCGCGAAG
101 CGCGCGGACA AATCGCGGCC GTCAAAGCGC AAACGACACG CGGCTGGCGG
151 AACACCGTCG AGCGCTGAC CGGCATCACG GAACGCGTCG CGAGGATTG
201 GGGCGTCTGT TCCCATCTCA ACTCGCTCGT CGACAGCGCC GAATCGCGG
251 CGCTCTATTA CGAAGTATG CTTGAAATCA CGGCTCTCTT CACCGAAATC
301 GGACAGACA TCGAATCTGA CACCGCTTC ARAACATCA AATATCCCC
351 CGAATTGCA ACGCTTCCC CCGCACAAA AACCAAGCTC GATCAGACC
401 TGGCGGATTT CGTATTGAGC GCGCGGAAC TGCCGCGCGA ACGCGAGCA
451 GAACCTGGCAA AACTGCAAAC CGAAGGCGCG CAACCTTCCG CCAATCTCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC ITTGACGATG
551 CGCAGCGCT TCGCGGCTT CCGGAAGACG CGCTGCCAT GTTTGCGCCG
601 GCGCGGCAA GCGAAGCAA AACAGGTTAC AAATCGCTT TGCAGATTCT
651 GCACTACCTT GCGCTTATCC AATACGCGG CAACCGGAAA CTGCGGAAAC
701 AATCTACCG CGCCTACGTT ACCCGTGCCA CGGAACCTTC AAACGACGCG
751 AATTTGACA ACACCGGCAA CATCGACCG ACCTCGAA ACGCATTGAA
801 AACCGCAA CTGCTCGCT TTAATAATTA CGCGGAATTC TCGCTGCAA
851 CCAAATGGC GGACAGCGCC GAACAGGTTT TAAACTCTCT GCACGACCTC
901 GCGCGCGCG CCAAACCTTA CGCGGAAAA GACCTCGCGG AAGTCAAAAG
951 CTTGCGCGC GAACACCTG GTCTCGCGCA CCGCGACGCG TGGGACTTGA
1001 GCTACGCGG CGAATAACTG CGCGAAGCCA AATACGATC CAGCGAAGAC
1051 GAAGTCARAA AATACTTCCC GTCTGCGCAA GTTCTGGCAG GCGTGTTCGC
1101 CCAATCAAAA AACTCTACG GCATCGGATT CGCGAAAAA ACCGTTCCCG
1151 TCTGCGCAA AGACGTGCGC TATTTTGAT TTGCAACAAA CGGCAAAAAC
1201 ATCGCGCGG TTATATGGA TTGTATGCA CGGCAAGGCA AACGCGCGG
1251 CGCTCGGATG AACCATACA AAGCGCGCG CCGCTTGGC GACGCGACG
1301 TGCAACTGCC CACCGCTAC CTGCTCGCA ACTTGCGCC GCGCGTGGC
1351 GGCAAGAAAG CGCGTTTAA GCGACGAGAA ATCTCTACCC TCTTCCACA
1401 AACCGGCCAC GACTGCACC ACCTGCTTAC CCAAGTGAC GAACGTGGC
1451 TCTCGCGCAT CAACGCGTA AAA

```

This corresponds to the amino acid sequence <SEQ ID 59; ORF 128-1.ng>:

```

g128-1.pep (partial)
  1 MIDNALLHLG EEPFRNQIKT EDIKPAVQTA IAEARGQIAA VKAQHTGTWA
  51 NTVERLTGIT ERVGRIWGVV SHLSVVDPD ELRAVYNELM PEITVFFTEI
 101 GQDIELYNRF KTIKNSPEFA TLPSPAKTKL DHDLRDFVLS GAELPPERQA
 151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
 201 AAQSEKTSY KIGLQIPHIL NVIQYAGNRE LREQIYRAYV TRASELSNDG
 251 KFDNTANIDR TLENALKTAK LIGFKNYAEI SLATKMADTP EQVNLFLHDL
 301 ARRAKPYAEK DLAEVKAFAF EHLGLADPOP WDLGYASEKL REAKYAFSET
 351 EVVKYFPVKG VLAGLFAQIK KLYGIGFAEK TVPVVHKDVR YFELQONGKT
 401 IGGVYMDLYA REGKRGGAWM NDYKRRRFA DGTLLQPTAY LVCNFAPPVG
 451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

m128-1/g128-1 ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa
overlap

      10      20      30      40      50      60
g128-1.pep MIDNALLHLGEEPRFNQIKTEDIKPAVQTAIAEARGQIAAVKAQHTGTWANTVERLTGIT
m128-1      MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAKAQHTGTWANTVEPLGIT
      10      20      30      40      50      60

      70      80      90      100     110     120
g128-1.pep ERVGRIWGVVSHLSVVDPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA
m128-1      ERVGRIWGVVSHLSVADTPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEF
      70      80      90      100     110     120

      130     140     150     160     170     180
g128-1.pep TLPSPAKTKLDHDLRDFVLSGAELPPERQAEALAKLQTEGAQLSAKFSQNVLDATDAFGIY
m128-1      TLPSPAKTKLNDHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY
      130     140     150     160     170     180

      190     200     210     220     230     240
g128-1.pep FDDAAPLAGIPEDALAMFAAAQSEKSGTKYKIGLQIPHILAVIQYAGNRELREQIYRAYV
m128-1      FDDAAPLAGIPEDALAMFAAAQSEKSGTKYKIGLQIPHILAVIQYADNRELREQIYRAYV
      190     200     210     220     230     240

      250     260     270     280     290     300
g128-1.pep TRASELSNDGKFDNTANIDRTLENALKTAKLLGFKNYAELSLATKMADTPPEQVNLFLHDL
m128-1      TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPPEQVNLFLHDL
      250     260     270     280     290     300

      310     320     330     340     350     360
g128-1.pep ARRAKPYAEKDIAEVKAFAREHLGLADPOPWDLGYASEKLREAKYAFSETEVKYFPVKGK
m128-1      ARRAKPYAEKDIAEVKAFARESLNLADLPWDLGYASEKLREAKYAFSETEVKYFPVKGK
      310     320     330     340     350     360

      370     380     390     400     410     420
g128-1.pep VLAGLFAQIKKLYGIGFAEKTVPVWHKDVRYFELQONGKTIGGVYMDLYAREGKRGGAWM
m128-1      VLNLGFAQIKKLYGIGFTEKTVPVWHKDVRYFELQONGETIGGVYMDLYAREGKRGGAWM
      370     380     390     400     410     420

      430     440     450     460     470     480
g128-1.pep NDYKRRRFADGTLLQPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD

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|||||
m128-1  NDYKGRRRFSDGTLQLPTAYLCNFAPFVGGRRLRSHDEILLFHETGHLHLHLLTQVD
              430      440      450      460      470      480

              490
g128-1.pep  ELGVSGINGVK
              |||||
m128-1      ELGVSGINGVWDVAVLPQSQMFNFVWEYNVLAQMSAHEETGVPLPKELFKMLAAKNFO
              490      500      510      520      530      540

```

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 60>:

```

a128-1.seq
1  ATGACTGACA  ACGCACTGCT  CCATTGGGCG  GAAGAAACCC  GTTTTGATCA
51  AATCAAAACC  GAAGACATCA  AACCAGCCCT  GCAAACGGCC  ATTGCCGAAG
101  CGCGCGAACA  AATCGCGGCC  ATCAAAGCCC  AAACGCGCAC  CGGCTGGGCA
151  AACACTGTGC  AACCCTGAC  CGGCATCACC  GAACGCGTGG  GCAGGATTTG
201  GGGCGTGGTG  TCGCACCTCA  ACTCGCTCAC  CGACAGCGCC  GAATCGCGCG
251  CGCGCTACAA  TGAATTATYG  CGCGAAATTA  CCGTCTCTCT  CACCGAAATC
301  GGACAAAGCA  TCGAGCTGTA  CAACCGCTTC  ARAACCTCA  AAACTCCGCC
351  CGAGTTGCAC  ACCCTCTCCC  ACGCGCAAAA  AACCAAACTC  AACCAAGATC
401  TCGCGCGATT  CGTCTCTACG  GCGCGGGAAC  TCGCGCCGCA  ACAGCAGGCA
451  GAATTGGCAA  AACTGCAAAAC  CGAAGCGCGC  CAACTTTCGG  CCAAAATCTC
501  CCAAAACGTC  CTAGACGCGA  CGACGCGGTT  CGGCATTATC  TTTGACGATG
551  CGCGACGCGT  TCGCGCGATT  CCGCAAGACG  CGCTCGCCAT  GTTTCGCGCT
601  GCGCGCGAAA  GCGAAGGCCAA  AACAGGCTAC  AAAATCGGTT  TGCAGATTTC
651  GCACTACCTC  GCGCTCATCC  AATAGCGCGA  CAACCGCAAA  CTGCGCGAAC
701  AAATCTACGG  CGCTACGCTT  ACCCGCGCCA  CGCGAGCTTC  AGACGACGGC
751  AAATTCGACA  ACACCGCAAA  CATGACCGCC  ACGCTCGAAA  ACGCGCTGCA
801  AACCGCAAAA  CTGCTCGGCT  TCAAAACTTA  CGCGGATTTG  TCGCTGCAAA
851  CCAAAATGGC  GGCACACCCC  GAACAAGTTT  TAAACTTCCT  GCACAGCTTC
901  GCGCGCGCGG  CCAAACCCTA  CGCGGAAAAA  GACCTCGCGC  AAGTCAAAGC
951  CTTGCGCGCG  GAAAGCGCTG  GCGTCCGCGA  TTTGCAACCG  TGGGACTTGG
1001  GCTACGCGCG  GCAAAACCTA  CGCGAAGCCA  AATACGCAAT  CAGCGAAACC
1051  GAAGTCAAAA  AATACTTCCC  CGTCCGCAAA  GTATTAAAGC  GACTGTTGCG
1101  CCAATCAAAA  AAACCTACAG  GCATCGGATT  TACCGAAAAA  ACCGTCCCGC
1151  TCTGCGCACA  AGACGTCGCG  TTGTATGGA  TTGTATGGA  TTTGTATGGA
1201  ATAGCGCGCG  TTATATGGA  TTGTATGGA  TTGTATGGA  TTGTATGGA
1251  CGCGTGGATG  AACGACTACA  AAGCGCGCGC  CGCTTTTTC  GACGCGACGC
1301  TGCAACTGCC  CACGCGCTAC  CTCGCTGCA  ACTTCACCCC  GCGCTCGCGC
1351  GGCAAAGAAG  CCGCGCTGAG  CCATGACGAA  ATCCTCACCC  TCTTCCACGA
1401  AACCGGACAC  GGCCTGACCC  ACCTGCTTAC  CCAAGTCGAC  GAACTGGGCG
1451  TATCCGGCAT  CAACGGCGTA  GAATGGGAGC  CAGTCGAATC  GCCAGTCAG
1501  TTTATGGAAA  ATTTGCTTTG  GAAATCAAT  GTCTTGGCGC  AAATGTCGCG
1551  CACGAGAA  ACCGCGCTTC  CCGTCCGAAA  AGAATCTTTC  GACAAATGCG
1601  TCGCGCGCAA  AACTCTTCCA  GCGCGAATGT  GATTACAGC  GACGAGAGAA
1651  TTCGCCCTCT  TTGATATGAT  GATTACAGC  GCGCGAATGT  GATTACAGC
1701  GAAAACTCGG  CAACAGGTTT  TAGACAGCGT  GACGAGAGAA  GTCGCGCTCG
1751  TCCGACCGCC  CGAATACAAC  CGCTTCCGCA  CACGCTTGGC  CACATCTTTC
1801  GCAGCGCGCT  ATTCCGCGAG  CTAATACAGC  TACGCTGGGG  CGGAAGTATT
1851  GAGCGCGGAC  GCATACGCGC  CTTTGAAGA  AAGCGACGAT  GTCGCGCGCA
1901  CAGGCAAAAC  CTTTGGCGAG  GAAATCTTCG  CGCTCGCGCG  ATCGCGACGC
1951  GCGGCGAAGT  CTTTCAAAGC  CTTCCGCGGA  CGGAAACGGA  GCATAGACGC
2001  ACTTTCGGCG  CACAGCGGCT  TGCACAACGC  GCGTTGA

```

This corresponds to the amino acid sequence <SEQ ID 61; ORF 128-1.a>:

```

a128-1.pep
1  MTDNALLHLG  EEPFRDQIKT  EDIKPALOTA  IAEAREQIAA  IKAQTHTGWA
51  NTEVPLTGTI  ERVGRIVGVV  SHINSVTDTP  ELRAAYNELM  FEITVFETEI
101  GODIELNYRF  KTIKSPEDF  TLSHAQKTKL  NHDRLDFVL  GAELPPEQQA
151  ELAKLTEGA  QLSAKFSQNV  LDATDAFGIY  FDAAPLACI  PEDALAMFA
201  AAQSEKGTGY  KIGLQIPHYL  AVIQYADNRK  LREQIYRAYV  TRASELSDDG
251  KFDNTANIDR  TLENALQTA  LLGFKNYAEL  SLATKMADTP  EQVNLFLIDL

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```

301 ARRAKPYAEK DLAEVKAFAR ESLGLADLQF WDLGYAGEKL REAKYAFSET
351 EVKKYFPVGG VNLGLFAQIK KLYGIGFTEK TVPVVHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAAM NDYKGRRRFS DGTLLQLPTAY LVCNFTPPVG
451 GKEARLSHDE ILTLFETHGH GLHLLTQVD ELGVSGINGV EWDAVEPLSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVBQME
551 FALFDMWYIS EDDEGLKQW QVLDVSRKE VAVVRPEPIN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAA*

```

m128-1/a128-1 ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

a128-1.pep	10	20	30	40	50	60
	MTDNALLHGEPRFDQIKTEDIKPALQTAIAEAREQIAAIAQTHTGWANTVEPLTGIT					
m128-1	MTDNALLHGEPRFDQIKTEDIKPALQTAIAEAREQIAAIAQTHTGWANTVEPLTGIT					
	10	20	30	40	50	60
a128-1.pep	70	80	90	100	110	120
	ERVGRINGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNREKTIKNSPEFD					
m128-1	ERVGRINGVVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNREKTIKNSPEFD					
	70	80	90	100	110	120
a128-1.pep	130	140	150	160	170	180
	TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSFPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
a128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLEQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKLEQIYRAYV					
	190	200	210	220	230	240
a128-1.pep	250	260	270	280	290	300
	TRASELSDDGKFDNTANIDRTLNALQTAKLGLGFKNYAEKLSLATKMDTPPEQVNLFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLNALQTAKLGLGFKNYAEKLSLATKMDTPPEQVNLFLHDL					
	250	260	270	280	290	300
a128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFARESLGLADLQFWDLYGAGEKLREAKYAFSETEVKKYFPVGG					
m128-1	ARRAKPYAEKDLAEVKAFARESLMLADIQFWDLYGASEKLREAKYAFSETEVKKYFPVGG					
	310	320	330	340	350	360
a128-1.pep	370	380	390	400	410	420
	VNLGLFAQIKKLYGIGFTEKTVPVVHKDVRVYFELQONGETIGGVYMDLYAREGKRGGAAM					
m128-1	VNLGLFAQIKKLYGIGFTEKTVPVVHKDVRVYFELQONGETIGGVYMDLYAREGKRGGAAM					
	370	380	390	400	410	420
a128-1.pep	430	440	450	460	470	480
	NDYKGRRRFS DGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFETHGHGLHLLTQVD					
m128-1	NDYKGRRRFS DGTLLQLPTAYLVCNFAPVGGKEARLSHDEILTLFETHGHGLHLLTQVD					
	430	440	450	460	470	480
a128-1.pep	490	500	510	520	530	540
	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ					

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```

m128-1      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLKPELFDKMLAAKNFQ
              490      500      510      520      530      540
              550      560      570      580      590      600
a128-1.pep  RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQVLDSVRKEVAVVRPPEYNRFANSFGHIF
              |||||
m128-1      RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQVLDSVRKKVAVIQPPEYNRFALSFHF
              550      560      570      580      590      600
              610      620      630      640      650      660
a128-1.pep  AGGYSAGYSYAWAEVLSDAYAAFEESDDVAATGKRFWQEILAVGSGRSAAESFKAERG
              |||||
m128-1      AGGYSAGYSYAWAEVLSDAYAAFEESDDVAATGKRFWQEILAVGSGRSAAESFKAERG
              610      620      630      640      650      660
              670      679
a128-1.pep  REPSIDALLRHSGFDNAAX
              |||||
m128-1      REPSIDALLRHSGFDNAVX
              670

```

206

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 62>:

```

m206.seq
1  ATGTTTCCCC CGACAAAC CCTTTCTCT TGTCTCAGCG CACTGCTCT
51  CGCCTCATGC GGCACGACCT CGGCAAAACA CCGCCACCG AAACCCAAAC
101 AGACAGTCCG GCAAAATCCAA GCGCTCCGCA TCAGCCACAT CGACCGACAC
151 CAAGGCTCGC AGGAATCAT GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GCGCGCAGCA GCACCCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAA AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCCCGCGACA TGGCGCGCGC AAGCCGAAA ATCCCAGACA GCGCGYTCAA
351 GGCCGCGGAC CTCGTATTCT TCAACAACCG CGCGCAGCAC CGCTACTCAC
401 ACGTCGGACT CTACATCGGC AACCGCGAAT TCATCCATGC CCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTT ACGCCAAAAA
501 CTACCTGGCG GCACATACCT TTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 63; ORF 206>:

```

m206.pep
1  MEPPDKTLFL CLSALLLASC GTTSGIHRQP KPKQTVRQIQ AVRISHIDRT
51  QGSOELMLHS LGLIGTPYKN GGSSTATGFD CSGMIQFVYK NALNVKLPRT
101 ARDMAAASRK TPDNRKAGD LVFFNTGGAH RYSHVGLYIG NGEFTHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFTPE

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 64>:

```

g206.seq
1  atgttttccc cgcacaaac ccttttctct tgtctcggcg cactgtctct
51  cgcctcatgc ggacagacct ccggcaaac cgcaccaacg aaacccaaac
101 agacagtcgc gcaaatccaa gcgctccgca tcagccacat cggcgccaca
151 caagctcgc aggaactcat gctccacagc ctcggaactca tcgcacgcc
201 ctacaaatgg gggcgcgaca gcacggcaac cggcttcgac tgcagcgga
251 tgattcaatt ggtttacaaa aagccctcaa acgtcaagct gccgcgaccc
301 gcccgcgaca tggcgcgccg caacccgaga atcccagaca gccgcctcaa
351 ggccggcgac atcgattctc tcaacacccg cggcgcaacac cgtactcaac
401 acgtcggact ctacatcggc aacggcgaa tcatccatgc ccccgcgagc
451 ggcaaaacca tcaaaaccca aaactctctc acacgctttt acgcacaaaa
501 ctaccttgga gcgcatacgt tttttacaga atga

```

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This corresponds to the amino acid sequence <SEQ ID 65; ORF 206.ng>:

```

g206.pep
1  MFSPDKTLFL CLGALLLASC GTTSGKHROP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQLVYK NALNVKLPR
101 ARDMAAASRK IPDSRLKAGD IVFFNTGGAH RYSHVGLYIG NGEFIHAPGS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

ORF 206 shows 96.0% identity over a 177 aa overlap with a predicted ORF (ORF 206.ng) from *N. gonorrhoeae*:

```

m206/g206
      10      20      30      40      50      60
m206.pep  MFPPDKTLFLCLLSALLLASC GTTSGKHROPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
g206      MFSPDKTLFLCLGALLLASC GTTSGKHROPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
      10      20      30      40      50      60
      70      80      90     100     110     120
m206.pep  LGLIGTPYKWGGSSTATGFD CSGMIQFVYK NALNVKLPR TAREDMAAASRK IPDSRLKAGD
g206      LGLIGTPYKWGGSSTATGFD CSGMIQFVYK NALNVKLPR TAREDMAAASRK IPDSRLKAGD
      70      80      90     100     110     120
      130     140     150     160     170
m206.pep  LVFFNTGGAHRYSHVGLYIGNGEFIHAPSGSGTKIKTEKLS TPFYAKNYLGAHTFFTE
g206      IVFFNTGGAHRYSHVGLYIGNGEFIHAPSGSGTKIKTEKLS TPFYAKNYLGAHTFFTE
      130     140     150     160     170

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 66>:

```

a206.seq
1  ATGTTTCCCC CGACAAAAC CCTTTCTCTC TGTCTCAGCG CACTGETCCT
51  CGCCTCATCG GGCACGACCT CCGGCAAAACA CCGCCACACG AAACCAACAC
101 AGACAGTCCG GCAAAATCCAA CGCGTCCGCA TCAGCCACAT CGACCGCAC
151 CAAGGCTCGC AGGAACATCAT GCTCCACAGC CTCGGACTCA TCGCGACGCG
201 CTACAAATGG GCGCGCAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAAA AACGCCCTCA ACGTCAAGCT GCGCGCAC
301 GCGCGCGACA TGCGCGCGCG AAGCGCGAAA ATCCCGGACA CGCGCTTAA
351 GCGCGCGGAC CTGCTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401 ACGTCGGACT CTAPPCGCGC AACGCGGAAT TCATCCATGC CCCAGCAGC
451 GCGCAAAACCA TCAAAACCGA AAAACTCTCG ACACGGTTTT ACCGCAAAA
501 CTACCTCGGC GCACATACCT TCTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 67; ORF 206.a>:

```

a206.pep
1  MFPPDKTLFL CLSALLLASC GTTSGKHROP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPR
101 ARDMAAASRK IPDSRLKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

m206/a206 ORFs 206 and 206.a showed a 99.4% identity in 177 aa overlap

```

m206.pep  MFPPDKTLFLCLLSALLLASC GTTSGKHROPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
a206      MFPPDKTLFLCLLSALLLASC GTTSGKHROPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
      10      20      30      40      50      60

```

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m206.pep	70	80	90	100	110	120
	LGLIGTPFKYKGGSSSTATGDCSGMIQVFYKKNALNVKLPRTARDMAAASKRPIDPSRKAGD					
a206	LGLIGTPFKYKGGSSSTATGDCSGMIQVFYKKNALNVKLPRTARDMAAASKRPIDPSRLKAGD					
	70	80	90	100	110	120
m206.pep	130	140	150	160	170	
	LVFNFTGGAHRYSHVGLYINGEFTHAPSSGKTIKTEKLSPTFYAKNYLGAHFTTFEX					
a206	LVFNFTGGAHRYSHVGLYINGEFTHAPSSGKTIKTEKLSPTFYAKNYLGAHFTTFEX					
	130	140	150	160	170	

287

The following partial DNA sequence was identified in *N. meningitidis* <SEO ID 68>:

[illegible]

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

m287.pep

1	MFKRSVIAMA	CIFALSACGG	GGGSGSPVKS	ADTLSKPAAP	VVSEKTEAK
51	EDAPQSGSGQ	QGAPSAQSQO	DMAAVSENT	GNGGVAATP	PKNEDEVAQN
101	IMFONAAAGTD	SSTPNPTDP	NMLAGNMEQ	ADTAGESSOP	AKPODMANNA
151	DGGMGGDDPSA	GGONAGNTAA	QTACQAGNQN	AGGSSDFIEA	SNFAPANGS
201	NFGRVGLDAN	VLIQDPSQNI	OLTHNGNENQ	SNAGSDPEEV	QLKSEFEKLS
251	DADKISNYKK	GGDKNQKFVGL	VADVSQVMGI	NOYIIFYPKK	PTSFARFERS
301	ARSRSLSLAE	MFLIPVNGAE	TLIVDGEAS	LTHSGSNIAP	PEGNYRFLAY

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351 GAEKLPGGSY ALRVQGEPAK GEMLAGAAYV NGEVLHFHTE NGRPYPTGR
 401 FAARKVDFGSK SVGDIIIDSG DLHMGTKQKF AAIDNGKFGD TWTENGSGDV
 451 SGKFYGPAGE EVAGKYSYRP TDAEKGFGV FAGKKEGD*

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 70>:

g287.seq
 1 atgttttaaac gcagtggtgat tgcaatggct tgtatttttc ccccttcacg
 51 ctgtggggggc ggcggtggcg gatcgcccgga tgtcaagctcg gcggacacgc
 101 cgtcaaaacc ggcgcgcccc ttgtttgctg aaaatgccgg ggaaggggtg
 151 ctgccgaaag aaaaagaaga tgaggaggcca gcggcggtg ccccgcaagc
 201 cgatacgcag gacgcacaag ccggggaagg cagccaagat atggcgggcag
 251 tttcggcaga aatacacagg aatggcggtg cggcaacaac ggacaacccc
 301 aaaaatgaag acgcggggcg gcaaatgat atgcgcgcaa atgcgcgcga
 351 atcgcgaat caaacaggga acaaccaacc gcgcggttct tcgatttcg
 401 ccccgcgctc aaacctctgc cctgcgaatg gcgtagcgga ttttggaag
 451 acgaaacgtg gcaattctgt tgtgattgac ggaccgtcgc aaaaataac
 501 gttgacccac tgtaaaaggcg attctgttaa tggtgataat ttttggaag
 551 aagaagcacc gtcaaaatca gaattgaaa aattaagtgga tgagaataaa
 601 attaaagcgc ataaaaaaga cgagcaacgg gagaattttg tcggtttggt
 651 tctgacagag gtaaaaaaag atggaaataa caaatataac atcttctata
 701 cggacaaacc acctactcgt tctgcacggt cgaggaggtc gcttcgggct
 751 gagattccgc tgattccctg caatcaggcc gatacgtgta ttgtgtagg
 801 ggaagcggtc agcctgacgg ggcattccgg caatatcttc gcgccgaag
 851 ggaattaccg gtatctgact tacggggcgg aaaaattgcc cggcggtatg
 901 tatgcctctc gtgtgcaagg cgaaaccggca aaaggcgaaa tgcttgttg
 951 caccgcccgt tacaacggcg aagtgtcgca ttccatagat gaaaacggcc
 1001 gtccgtaccc gtccggaggc aggtttgccg caaaagtcga ttccgcgac
 1051 aactctgtgg acggcattat cgacacggcg gatgatttgc atatgggtac
 1101 gcaaaaattc aaagcgcgca tcatgggaaa cggctttaa gggacttgg
 1151 cggaaaatgg cggcggggat gtttcggaaa ggtttacgg ccggcgccgc
 1201 ggaagaatgg cgggaaaata cagctatcgc ccgacagatg ctgaaaaggg
 1251 cggattcggc gtgtttgccg gcaaaaaga tcgggattga

This corresponds to the amino acid sequence <SEQ ID 71; ORF 287.ng>:

g287.pep
 1 MFKRSVTJAMA CIFPLSACGG GGGGSPDVKS ADTPSKPAAP VVAENAGEV
 51 LPKEKKDEEA AGGAPOADTQ DATAGEGSDQ MAAVSAENTG NGGAATTDNP
 101 KNEDAGAQND MPQNAAESAN QTGNQFPAGS SDSAPASNPA PANGGSDFR
 151 TNVGNVVID GPSONITLTH CKGDSNCGDN LLDEEAPSKS EFEKLSDEEK
 201 IKRYKQDQR ENFVLVADR VKKDGNTKYY IFYDQPPTR SARSRSLPA
 251 EIPLIPVNQA DTLIVDGAU SLTGLSHNIF APEGNVLTL YGAERLPGGS
 301 YALRVQGEPA KGEMLVGTAV YNGEVLHFHM ENGRFYPGGG RFAAKVDFG
 351 KSVGDIIIDSG DDLHMGTKQF KAAIDNGKFK GTWTEGGGD VSGRFYGPAG
 401 EEVAGKYSYR PTDKKGFGV VFAGKKDRD*

m287/g287 ORFs 287 and 287.ng showed a 70.1% identity in 499 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGSPDVKSADTLSKPAAPVVSE-----	KETE			
g287					
	10	20	30	40	50
	MFKRSVIAMACIFALSACGGGGGSPDVKSADTFSKPAAPVVAENAGEVLPKEKKDEEA				
	10	20	30	40	50
m287.pep	50 KEDAPQAGSQGGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGT				
g287					
	AGGAPQADTQ--ATAGEGSDMAAVSAENTGNGGAATTDNPKNEDEVAQNDMPQNA--				
	70	80	90	100	110

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```

110      120      130      140      150      160      169
m287.pep DSSTFNHTFDPNMLAGNMENQATDAGESSQPANQPDHANAADGMQGGDPSPAGGQNAGMTA
g287
-----
170      180      190      200      210      220      229
m287.pep AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFRVLDLANGVLIDGPSQNIITLTHCKGDS
g287      -ESANQTGNNQFAGSSDSAPASNPAPANGGSDFGRTNVGNSVVIDGPSQNIITLTHCKGDS
120      130      140      150      160      170
230      240      250      260      270      280      289
m287.pep CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDGNKDFVGLVADSVQMKGINQYIIFYKP
g287      CNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFGVLVADRVKKGDTNKYIIFYTD
180      190      200      210      220      230
290      300      310      320      330      340      349
m287.pep KPTSFARFRRSARSRRSLPAEMPLIPVNOADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT
g287      KPFT-----RSARSRRSLPAEIPLIPVNOADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT
240      250      260      270      280      290
350      360      370      380      390      400      409
m287.pep YGAEKLPGGSYALRVQGEPAKGEMLAGAAVTVNGEVLHFTENGRRPYPTGRGFAAKVDFGS
g287      YGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFTENGRPYPSGGRFAAKVDFGS
300      310      320      330      340      350
410      420      430      440      450      460      469
m287.pep KSDGIIIDSGDDLHMGTQKFAAIDGNGFKGTWTENGSGDVSQKGFPGAGEEVAGKYSYR
g287      KSDGIIIDSGDDLHMGTQKFAAIDGNGFKGTWTENGSGDVSQKGFPGAGEEVAGKYSYR
360      370      380      390      400      410
470      480      489
m287.pep PTDAEKGGFVGFAGKKEQDX
g287      PTDAEKGGFVGFAGKKEQDX
420      430

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287.seq
1  ATGTTTAAC  CGAGTGTGAT  TGC AATGGCT  TGTATTGTTG  CCGTTTCAGC
51  CTGTGGGGCC  GCGGTGGCG  GATCGCCCGA  TGTTAAGTCC  GCGACACGCG
101  TGTCAA AACC  TGCGCCCOCT  GTTGT TACTG  AAGATGTCGG  GGAAGAGGTT
151  CTGCCGAAG  AAAAGAAAGA  TGAGGAGCGC  GTGATGTGTT  CGCCGCAAGC
201  CGATACGAG  GACGCAACCG  CCGGAAAAGG  CGGTCAAGAT  ATGGCGCGAG
251  TTTCCGCAGA  AATACAGGC  AATGGCGGTG  CGGCACACAC  GGATATCCCG
301  GAAAATAAAG  ACGAGGGACC  GCAAAATGAT  ATGCCGCAAA  ATGCCGCGCA
351  TACAGATAGT  TCGACACCGA  ATCACACCCC  TGCACCGAAT  ATGCCAACCA
401  GAGATATGGG  AAACCAAGCA  CCGGATGCGG  GGAATTCGCG  ACAACCGGCA
451  AACCAACGG  ATATGGCAAA  TCGCGCGGAC  GGAATGCAGG  GGGACGATCC
501  GTCGCAGGG  GAAATATCCG  GCAATACGGC  AGATCAAGCT  GCAATCAAG
551  CTGAAAACAA  TCAAGTCGGC  GGCCTCAAAA  ATCCTGCTCC  TTCAACCAAT
601  CCTAACGCCA  CGAATGGCGG  CAGCGATTTT  GGAAGGATAA  ATGATAGCTAA
651  TGGCATCAAG  CTGACAGCG  GTTCGGAAAA  TGTAACGTTG  ACACATTGTA
701  AAGACAAAGT  ATGCGATAGA  GATTTCCTTAG  ATGAAGAAAG  ACCACAAAAA
751  TCGAATTTG  AAAAATTAA  TGATGAAGAA  AAAATTAA  AATATAAAAA
801  AGACAGCA  CGAGAGAA  TTGTCGGTT  GGTTCGTGAC  AGSGTAGAAA

```

```

m287.pep      1  MFKRSVIAMAC  CIVALSSACGG  GGGGSPOVKS  ADTLSKPAAP  VVTEVDGEEV
    51  LPKEKKDEEA  VSGAPQADTQ  DATQACGGQD  MAAVSAENTG  NNGGAATTDNP
   101  ENKDEGPOND  MPQNAADTDS  STPNHTPAPN  MPTRDMGQQA  PDAGESAQA
   151  NQPDMANAAD  CMGQDDFSAQ  ENAGNTADAQ  ANQANENNVO  GSONPASSTN
   201  PNATNGSGSF  GRINVANGEK  LDSGSENVTL  THCKDKVCDR  DFLDEEAPPK
   251  SEFEKLSDSE  KINKYKQDE  RENFYCJAD  RVEKNGNTRY  VLIYKDKSAS
   301  SSSARFRRSA  RSRRLSPAE  PLVPVQNAQT  ELGTACTAVN  YLGHSGNIFAP
   351  EGNRYRLTY  AEKLSGGESA  VLSVCEPAKG  ELMACTAVYN  YLGHSHFMEW
   401  GRPSSSGGRF  AAKVDFGSKS  VGGIISGDD  LHMGTKRQFA  VIDNGNFKGT
   451  WTENGGGDVS  GRFYGPAGEE  VAGKYSVRT  DAEKGGGVF  AGKQEQA*

m287/a287      ORFs 287 and 287.a showed a 77.2% identity in 501 aa overlap

              10      20      30      40      49
m287.pep      MFKRSVIAMACIFALSACGGGGGSPDVKSADTLSKPAAPVSE-----KETEA
              |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
a287           MFKRSVIAMACIVALSACGGGGGSPDVKSADTLSKPAAPVVTEVDGEEVLPKEKKDEEA
              |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
              50      60      70      80      90      100      109
m287.pep      KEDAPAGSGQGQGAPSAQSGSDMAAVSEENTNGGAVTADNPKNDEVAQNDMPQNAAGT
              ||||  ||  || :||:||||:||||:||||:||||:||||:||||:||||:||||:||||:
a287           VSGAPQADTQ--DATAGCGQGDMAAVSAENTNGGGAATTDPNPNKDEGPONDMPQNAADT
              70      80      90      100      110
              110     120     130     140     150     160     169
m287.pep      DSSTPNHPTFPNNLHLAGMNEQATDAGESOSPAPQPDMANAADMCGQDDPSAGGQAGNATA
              |||||  |||  || :||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
a287           DSSTPNHPTPAPNMPTRDMGQQAADPAGESAQPDAPMANAADMCGQDDFSAQ-ENAGNTA
              120     130     140     150     160     170
              170     180     190     200     210     220     229
m287.pep      AQGANAGQNVNAAQSSSDPTPASNPAPAGGSMFGRVDLANGVLIDGPSQNTLTHCKGDS
              |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
a287           DQANQAENNVOVGSGSQNPASSTPNATNGSGDFGRINVANGIKLDSGSENVTLTHCKDKV
              180     190     200     210     220     230
              230     240     250     260     270     280     289
m287.pep      CSGNNFLDEVQLKSEFEKLSDSADKISNYKDKGNKDKEVLGVADSVMGKGINQYIIFRYP
              |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
a287           CD-RDFLDEEAAPKSEFEKLSDSEKINKYKDEQRENFVLGVADRVKNGNTKNYIYKD
              240     250     260     270     280     290
              290     300     310     320     330     340
m287.pep      KP--TSFARFRRSARSRSRLSPAEMLPLVPVQADTLVIDGEAVSLTGHSGNIFAPENGVRY

```

4066.pep		Sequence	Peptide	Score	SEQUEST	75, ORF 4007	
1	1	MOARLLIPIL	FVSFILLSACG	10.2	TLTGIPSHGG	KRKFVAFGEI	VASARAARVET
51	1	DDMLQALHGR	KVALYLIATNG	10.2	DGGSSGLTGR	PYLSIDALRG	VGSASPAVTK
101	1	DYDTPRYRHT	KATSSGGLTG	10.2	LTSLSLTMA	PAISRTQSDG	SSASASGLNL
151	1	IGGMSGGDYRNE	TLTINPRDTA	10.2	FLKSLQVOTF	FLRGIDVVSF	ANADTVDFR
201	1	IDVFQITGRNR	TEMLHLYNAET	10.2	LNAQTKLEYF	AVDRNTKUPS	IKPKTNKATL
251	1	AYKENYALWM	GVFYKSGKIG	10.2	PTEGLMVDFS	DIRPYGMHTG	NSAPSVEADN
301	1	SHEGYGYSDE	VYVROHROGR	10.2	*		

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

```

g406.seq
1  ATGCGGGCAC  GCGTGTGAT  ACCATTCTT  TTTTCAGTT  TTTATTTATC
51  CGCCTCGGG  ACACATGAC  GTATTCATC  GCATGGCGG  GGCAAACGCT
101 TCGCGGTGGA  ACAAGAACT  GTGGCCGCT  CTGCCAGAG  TGCGTTAAA
151 GACATGGATT  TACAGGCAT  ACACGGACG  AAAGTTGAT  TGTACATTGC
201 AACTATGGGC  GACCAAGGT  CAGGCAATT  GACAGGGGT  CGCTACTCCA
251 TTGATGCACT  GATTGCGGC  GAATACATA  ACAGCCCTG  CGTCCGCC
301 GATTACACCT  ATCCGCGTA  CGAAACCAC  GCTGAAACA  CATCAGCGCG
351 TTTGACGGG  TTAACCACT  CTTTATCTA  ACTTAATGC  CCTGCACCT
401 CGCGCACCCA  ATCAGACGT  AGCGGAAGT  GGAGCAGTC  GGCCTTAAAT
451 ATTGSCGGG  TGGGGGATTA  TCGAAATGA  ACCTTGACG  CCAACCCGCG
501 CGACACTGCC  TTTCTTCCC  ACTTGGTGA  GACCGTATT  TTCTGCGCG
551 GCATAGACGT  TGTCTCTCT  GCCAATGCC  ATACAGATG  GTTTATTAA
601 ATCGACGTAT  TCGAACGAT  ACGCAACAG  ACCGAAATG  ACCTATACAA
651 TGCCGAACA  CTGAAGCC  AACCAAACT  GGAATATTC  GCAGTAGACA
701 GAACCAATA  AAAATTGCT  ATCAAACTA  AAACCAATG  GTTTGAAGCT
751 GCCTATAAG  AAAATTACG  ATTGTGGAT  GGCGCGTAT  AAGTAGCAA
801 AGGAATCAA  CCGACGGA  GATTGATGG  CGATTCTCC  GATATCCAC
851 CATACGCCA  TCATACGGT  AACTCCGCC  CATCCGTAG  GGCTGATAAC
901 AGTCATGAG  GGTATGGAT  CAGCGATGA  GCAGTGGCA  AACATAGACA
951 AGGGCAACCT  TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

```

g406.pep
1  MRARLLIPI  LFSVFILS  SAGTTLTG  IPIPSHGG  GKRFVEQE  LVAASARA  VVKMDLQALHGR
51  DMDLQALHGR  KVALYIAT  MG DQSGSL  TGRYSIDAL  IRGEYINS  PAVRTDYTY  PRYETTAE
101  DYTYPRYETT  AETTSGL  TGLTTSLS  TLNAPALSR  TQSDGSG  RSSLGLN
151  IGGMDYRNE  TLTNPRDT  AFLSHLVQ  TVFLRGID  VVSPANAD  TDFVFIN
201  IDVFGTIRN  RTEMHLYA  ET LKAQTK  LEYFVDR  TRNKLKLL  IKPKTNAF  EA
251  AYKENYALW  MGPYKSK  GKIPTEGL  MVDFSDI  QPYGNHTG  NSAPSVEAD  N
301  SHEGYGSDE  AVRQHRQ  GQP *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

```

g406/m406
10 20 30 40 50 60
g406.pep MRARLLIPI LFSVFILSAGTTLTG IPIPSHGGKRFVEQELVAASARA VVKMDLQALHGR
m406 MQARLLIPI LFSVFILSAGTTLTG IPIPSHGGKRFVEQELVAASARA VVKMDLQALHGR
10 20 30 40 50 60
70 80 90 100 110 120
g406.pep KVALYIATMGDQSGSLTGG RYSIDALIRGEYINS PAVRTDYTY PRYETTAE TTSGLTGT
m406 KVALYIATMGDQSGSLTGG RYSIDALIRGEYINS PAVRTDYTY PRYETTAE TTSGLTGT
70 80 90 100 110 120
130 140 150 160 170 180
g406.pep LTTSLSLTNAPALSR TQSDGSGSRSS LGLNIGGMDGYRNETLT TNPRDTAFLSHLVQTVF
m406 LTTSLSLTNAPALSR TQSDGSGSKSS LGLNIGGMDGYRNETLT TNPRDTAFLSHLVQTVF
130 140 150 160 170 180
190 200 210 220 230 240

```

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```

g406.pep      FLRGIDVVSANADTVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
                |||
m406          FLRGIDVVSANADTVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
                |||
                190      200      210      220      230      240

                250      260      270      280      290      300
g406.pep      IKPKTNAFEAAKYENALWMGPYKVSIGI KPT EGLMVD FSDIOPYGNHTGNSAPSV EADN
                |||
m406          IKPKTNAFEAAKYENALWMGPYKVSIGI KPT EGLMVD FSDIOPYGNHTGNSAPSV EADN
                |||
                250      260      270      280      290      300

                310      320
g406.pep      SHEGYGYSDEAVRQHRQGQPX
                |||
m406          SHEGYGYSDEVVRQHRQGQPX
                |||
                310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1  ATCGAAGCAC  GGCTGCTGAT  ACCTATTCTT  TTTTCAGTTT  TTAATTATCT
51  CGCCTCGGGG  ACACGTGACG  GTATTCCATC  GCATGGCGGA  GGTAAACGGT
101  TCGCCGCGCA  ACAGACACTT  GTGGCCGCTT  CTGCCAGAGC  TGCCGTATAA
151  GACATGGATT  TACAGGCATT  ACACGGACGA  AAAGTTGCAT  TGTAAGTGGC
201  AACTATGGCG  GACCAAGGTT  CAGGCAGTTT  GACAGGGGGT  CGCTACTCCA
251  TTGATGCACT  GATTTCGTGC  GAATACATAA  ACAGCCCTGC  CGTCCGTACC
301  GATTACACCT  ATCCACGTTA  CGAAACCACC  GGTGAACAA  CATCAGGCGG
351  TTTGACAGGT  TTAACCACTT  CTTTATCTAC  ACTTAATGCC  CCTGCATCTC
401  CGCGCACCCA  ATCAGACGGT  AGCGGAAGTA  AAAGCAGTCT  GGGCTTAAT
451  ATTGGCGGGA  TGGGGGATTA  TCGAAATGAA  ACCTTGACGA  CTAACCGCGG
501  CGACACTGCC  TTTCTTCCC  ACTGTGTACA  GACCGTATT  TTCTGCGGCG
551  GCATAGAGCT  TGTTCCTCCT  GCCAATCCCG  ATACGAGTGT  GTTTATTAA
601  ATCGACGTAT  TCGGAACGAT  ACCCAACAGA  ACCGAAATGC  ACCTATACAA
651  TGCCGAACAA  CTGAAGCCCT  AAACAAACT  GGAATATTTC  CGAGTAGACA
701  GAACCAATAA  AAAATTGCTC  ATCAAAACAA  AAACCAATGC  GTTTGAAGCT
751  GCCTATAAAG  AAAATTACGC  ATTGTGGATG  GGACCGTATA  AAGTAAGCAA
801  AGGAATATAA  CCGACAGAAG  GATTAAATGT  CGATTCTTCC  GATATCCAAC
851  CATACGGCAA  TCATATGGGT  AACTCTGCCC  CATCGTAGA  GGCTGATAAC
901  ACTCATGAGG  GGTATGGATA  CAGCGATGAA  CGAGTGGCAC  GACATAGACA
951  AGGGCAACCT  TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1  MQARLLIPIL FSVFILSACG  TLTGIPSHGG  GKRFAVEQEL  VAASARAQVK
51  DMDLQALHGR  KVALYIATMG  DQSGSLTGG  RYSIDALIRG  EYINSPAVRT
101  DYTYPREYET  AETTSGLLTG  LTSLSLTSL  PALSRQSDG  SGSKSSGLLN
151  IGGMGDYRNE  TLTNPRDTA  FLSHLVQTV  FLRGIDVVS  ANADTVFIN
201  IUVFGTIRNR  TEMHLYNAET  LKAQTKLEY  FAVDRTNKK  IKPKTNAFE
251  AYKENALWM  GPYKVSIGI  PTEGLMVD  FSDIOPYGN  HMGNSAPSV EADN
301  SHEGYGYSDE  AVRRHRQGQ  P

```

m406/a406 ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

```

                10      20      30      40      50      60
m406.pep      MQARLLIPILFSVFILSACGTLTGIPSHGGKRF AVEQELVAASARAQVKDMDLQALHGR
                |||
a406          MQARLLIPILFSVFILSACGTLTGIPSHGGKRF AVEQELVAASARAQVKDMDLQALHGR
                |||
                10      20      30      40      50      60

                70      80      90      100     110     120
m406.pep      KVALYIATMGDQSGSLTGG RYSIDALIRGEYINSPAVRTDYTYPRYETTAETTSGLLTG
                |||

```

WO 00/66791

PCT/US00/05928

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```

a406      KVALYIATHSGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTGTG
           70      80      90      100     110     120
m406.pep  130      140      150      160     170     180
           LTTSLSLTINAPALSRQTQSDGSGKSSSLGNIIGMGDYRNETLTNPRDTAFLSHLVQTTF
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
a406      130      140      150      160     170     180
           LTTSLSLTINAPALSRQTQSDGSGKSSSLGNIIGMGDYRNETLTNPRDTAFLSHLVQTTF
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
m406.pep  190      200      210      220     230     240
           FLRGIDVVS PANADTDVFINIDVFGTIRNRNTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
a406      190      200      210      220     230     240
           FLRGIDVVS PANADTDVFINIDVFGTIRNRNTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
m406.pep  250      260      270      280     290     300
           IKPKTNAFEAAAYKENYALWMGPYKVKSGIKPTEGLMVFSDIIRPYGNHTGNSAPSV EADN
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
a406      250      260      270      280     290     300
           IKPKTNAFEAAAYKENYALWMGPYKVKSGIKPTEGLMVFSDIIPYGNHMCNSAPSV EADN
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
m406.pep  310      320
           SHEGYGYSDEVVRQHRQGQPK
           ||||||||||||||||||
a406      310      320
           SHEGYGYSDEAVRRHRQGQPK
           ||||||||||||||||||

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

```

m726.seq
1  ATGACCATCT  ATTTCAAAAA  CGGCTTTTAC  GACGACACAT  TGGCGCGCAT
51  CCCGCAAGGC  GCGGTTGCGG  TCCGCGCGGA  AGAATACGCC  GCCCTTTTGG
101 CAGGACAGGC  GCAGGGCGGG  CAGATTGCGG  CAGATTCGGA  CGGCGCGCCC
151 GTTTTAAACC  CGCCGCGCCC  GTCCGATTAC  CAGCAATGGG  ACGGCAAAAA
201 ATGGAATATC  AGCAAAGCCG  CGCGCGCGCG  CGGTTTCGCG  AAACAAAAAA
251 CGGCCTTGCG  ATTCGCGCTC  GCGGAAAGAG  CGGACGAATC  CAAAAAACAGC
301 CTCTTGCGGG  GCTATCCCCA  AGTGGAAATC  GACAGCTTTT  ACAGGCAGGA
351 AAAAGAGGCC  CTGCGCGCGC  AGGCGGACAA  CACGCCCGCG  ACCCGATGTC
401 TGGCGCAJAT  CGCGCGCGCA  AGGCGCGTGG  AATGGACACT  TTGATTGAA
451 AAGATTATCG  AAAAATCCGC  CGGCTGGCT  GTTCGCGCGG  CGCGGATAT
501 CGGAAACGCT  CAGCAGCTCG  AAGACAAATT  GAACACCATC  GAAACGCGCG
551 CGGATTGGA  CGCGCTGGAA  AAGAAATCG  AAGATGGAC  GCTAACATC
601 GGCTGA

```

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

```

m726.pep
1  MTIYFKNGFY  DPTLGGIPEG  AVAVRAEAYA  ALLAGQAQGG  QIAADSDGRF
51  VLTTPRPESFY  HEWDGKKWKI  SKAAAAARFA  KQKTALAFRL  AEKADELKNS
101 LLAGYPOVEI  DSYFYRQEKA  LARQADNNAP  TPMLAQIAAA  RGVELDVLIE
151 KVIKESARLA  VAAGAIIGKR  QLEDEKLNTI  ETAPGLDALE  KEIEENTLINI
201 G*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 82>:

```

m907-2.seq
1  ATGAGAAATC  CGACCGATAC  CCTACCGGTT  AATCTGCAAC  GCCCGCGCCT
51  GTTGTGTGCC  GCCGTCGCGT  TGTTGCTCAG  TCCTTGCGCG  CACGCGCGCG
101 CGCAACGTGA  GGAAACGCTT  GCCGACGATG  TGGCTTCGCT  GATGAGGAGT

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```

151 TCTGTGGGCA GCGTCAATCC GCGAGGCGTG GTGTTTGACA ATCCGAAAGA
201 GGGCGAGCGT TGGTGTGCTG CCAATGTGGC ACGTTTGGCA AGGTTCTGTC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGCTGG TCAATATCCA GTACGAAAGC
301 AGCGGGGCGG GTTGGATAC GCAGATTGTG TTGGGGCTGA TTGAGGTGGA
351 AGCGGGCTTC GCGCAGTATG CAATCACGGG TCGCGGCTGA CCGGCGCTGA
401 TGCAGGTTAT GCCGTTTTCG AAAAANTACA TCGCAAAACC GCGCACAAAC
451 CTGTTCGACA TCCGCACCAA CTCTGGTTAC GGCTGTACCA TCCTGCGCCA
501 TTACCGGAAT CTGAAAAGG GCAACATCGT CCGCGCGCTT GCCCGCTTTA
551 ACGGAGCTT GGGCAGCAAT AATATATCGA ACGCGGCTTT GGGCGCGTGG
601 CGCAACCGCT GGCAGTGGCG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

m907-2 . pep

```

1 MRKPTDTLFV NLQRRRLCA AGALLSPLA HAGAOREETL ADDVASVMRS
51 SVGSVNPRL VFDNPKGER WLSAMSARIA RFVPEEEERR RLLVNIQYES
101 SRAGLDTQIV LGLLEVESAF RQYASGVGA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNLRY GCTILRHYRN LEKGNIVRAL ARFNGSLGSN KYPNAVILGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

m953 . seq

```

1 ATGAAAAAAA TCATCTTCGC CGCACTCGCA GCGCGCGCCA TCAGTACTGC
51 CTCGCGCGCC ACCTACAAGT TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTTCAACACC AGCACCAACG TCGCGGTTTT TTACGGTCTG
151 ACGGGTTCCG TCGAGTTGCA CCAAGCAAAA CGCGACGGTA AAATCGACAT
201 CACATTCGCC ATTGCCAACC TGCAGACGGG TTGCGACAC TTTACGACAC
251 ACCTGAAATC AGCCGACATC TTGATGCGCG CCAATATACC GGACATCGCG
301 TTTGTTTCCA CCAAAATCAA CTTCACCGCG AAAAACTGG TTTCGGTTGA
351 CGGCAACCTG ACCATGCAAG GCAAAACCGC CCGCGTCAA CTCAAAGCGC
401 AAAAATTCAA CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTTGTGGC
451 GCGGACTTCA GCACACCAT CGACCGCACC AAATGGGGCA TGGACTACCT
501 CGTTAAGCTT GGTATGACCA AAAGCGTCGG CATCGACATC CAAATCGAGG
551 CAGCCAACCA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

m953 . pep

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHNT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIP IANLQSGSQH FTDHLKSADI FDAAQYPDIR
101 FVSTKFNENG KKLVSVDGLN TMHGKTAPVK LKAERKFNQY SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GWKSVRIDI QIEAAQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

orf1-1 . seq

```

1 ATGAAAAACAA CCGACAAAGC GACAACCGAA ACACACCGGA AAGCCCGGAA
51 AACCGCGCCG ATCCGCTTCT GCGCTGCTTA CTTAGCCATA TGCCTGCTGT
101 TCGCATTTCT TCCCAAGCC TGGCGGGGAC ACATTATATT CGGACATCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAT AAAGGCAAGT TTGCACTCGG
201 GGCGAAAGAT ATTGAGGTTT ACAACAAAAA AGGGAGGTTG GTGCGCAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGTTGTC CGGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCTGG CACATAACGG
351 CGGCTATAAC AACGTTGATT TTGGTGGCGA AGGAAGAAAT CCGGATCAAC
401 ATCGTTTAC TTATAAAAAT GTGAAACGGA ATAATATATA AGCAGGAGT
451 AAAGGCAATC CTTATGGCGG GATATATCAT ATGCCGGGTT TGCATATATT
501 TGTACAGAT GCAGAACCTG TTGAATGAC CAGTTATATG GATGCGCGGA

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o

551 AATATATCGA TCAAAATAT TACCCTGACC GTGTCGTAT TGGGGCAGGC
 601 AGGCAATATT GGCATCTGA TGAAGATGAG CCCAATAACC GCGAAAGTTC
 651 ATATGACCT ATCTGTGGT CGTGGTGGC ANTAGCTTTG ANTAGCTTTG
 701 CACAAATATG ATCAGCTGGT GGCACAGTCA ACTTAGGTAG TGAANAATTT
 751 AAACATAGCC CATATGGTTT TTTACCAACA GGAGGCTCAT TTGGGCACAG
 801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAGGCAAAAG TGGTTAATTA
 851 ATGGGGTATT GCAAACGGGC AACCCCTATA TGGAAAAGAG CAATGGCTTC
 901 CAGCTGGTTC GTAAGATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC
 951 CCATTACGTA TTCTACGAAC CACGTCAAAA TGGGAATAC TCTTTAACG
 1001 ACGATATATA TGGCACAGGA AAAATCAATG CCAAAATATG ACNCAATTCT
 1051 CTGCTTATA GHTTAAAAAC ACGACCGCTT CATTGTTTAT ATGTTCTTTT
 1101 ATCCGAGACA CGAAGAGAAC CTGTTTATCA TCGTCAGGT GGTGTCAACA
 1151 GTTATCTGACC CAGACTGAAT AATGGAGAAA ATATTCTCTT TATTGACGAA
 1201 GGAANAAGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAGG
 1251 ATTATATTTT CAAGGAGATT TTACGCTCTC GCCTGAAAT AACAAGAACT
 1301 GGCAAGGCGC GGGCGTTTAT ATCAGTGAAG ACAGTACCGT TACTTGGAAA
 1351 GTAACGCGCG TGCCAAACGA CCGCTGTCTC AAAATCGGCA AAGGCACGCT
 1401 GCACGTTCAA GCCAAGGGGG AAAACCAAGG CTCGATCAGC GTGGGCGAGC
 1451 GTACGTCAT TTTGATCAG CAGCAGACG ATAAAGCACA AACAACAAGC
 1501 TTTAGTGAAA TCGCTTGTGT CAGCGCGAGG GGTACGCTGC AACTGAATCG
 1551 CGATAATCG TTCAACCCCG ACAAACTCTA TTTGCGCTTT CGCGCGGAC
 1601 GTTTGGATTT AAACGGGCAT TCGCTTGTGT TCCACCGTAT TCAAAATACC
 1651 GATGAAGGGG CGATGATTGT CAACCAAT CAAGACAAG AATCCACCGT
 1701 TACCATTACA GGCATAAAG ATATTGCTAC AACCGGCAAT AAACAACAGT
 1751 TGGATAGCRA AAAAGAAATT GCCTACAAG GTTGGTTTGG CGAGAAGAT
 1801 ACAGCCAAAA CGACGCGGCG GCTCAACTCT GTTTACAGC CGCGCGCAGA
 1851 AGACGACACC CTGCTGCTTT CGCGCGGAC AAATTTAAC GGCATGATCA
 1901 CCGAAAACAA CGCAAACTGT TTTTTCAGG CGAGACCAC AACCGACGCC
 1951 TACAATCAIT TAAACGACCA TTGCTGCAA AAGAGGGCA TTCTCGCGG
 2001 GGAATCGTG TGGGACACG ACTGGATCAA CGGCACATT AAAGCGGAAA
 2051 ACTTCCAAT TAAAGCGGGA CAGGCGGTGG TTTCCGCAA TGTTCGAAA
 2101 GTGAAGGCGC ATTGGCATT GAGCAATCAC GCCCAAGCAG TTTTGTGTGT
 2151 CGCACCGCAT CAAAGCCACA CAATCTGTAC ACGTTGGAGC TGGACGGGTC
 2201 TGACAAATTG TGTGAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA
 2251 TTGACTAGA CGGACTCAG CGCAATCGC GATCTTGCG ATACGCTCA
 2301 TTTAATCTC ACAGGGCTTG CCACACTCAA CGCAATCTT AGTGCAAATG
 2351 GCGATACAGC TTATACAGTC AGCCACAAGC CCACCCAAA CGGCAACTTT
 2401 AGCCTCGTGG GCAATGCCCA AGCAACMTT AATCAAGCCA CATTAAACGG
 2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGCG
 2501 TACAAAACGG CAGCTGACG CTTCGCGCA ACGCTAAGC AAACGTAAGC
 2551 CATTCCGAC TCAACGCTAA TGCTCCCTA GCGGATAGG CAGTATTCCA
 2601 TTTTGAAAGC AGCGCTTTA CGCGACAAAT CAGCGCGCGC AAGGATACGG
 2651 CATTACACTT AAAGGACAG GATTTGACG GATCCGTGAG CAGCAATTA
 2701 GGCATTATA ACCTTGACRA CGGCACCTT ACACTCAAT CCGGCTATCG
 2751 CCACGATGCG CAGGGGCGCG AAACCGGAGC TGGCAGAGAT CGCGCGCGCC
 2801 GCGGTTTCGCG CGGTTGCGCG CGTTCCTAT TATCCGTTAC ACOCGCACT
 2851 TCGGTAGAAT CCGTTTCAA CAGCTGACG GTAACGGCA AATTGAACGG
 2901 TCAGGGACAA TTCCGCTTTA TGTGCGAAT CTTCGCTAC CGCAGCGACA
 2951 AATTGAAGCT GCGCGAAAGT TCCGAAGGCA CTACACCTT GCGGCTCAAC
 3001 AATACCGGCA ACGAACCCTG AAGCCTCGAA CAATTGACG TAGTGGAAAG
 3051 AAAGACACAC AAACCGCTGT CCGAARACT TATTTCAC CTGCAAAAG
 3101 ACACACTCGA TGCCGGGCGG TGGGTTTACC AACTCATCCG CAAGACGGCG
 3151 GAGTTCCGCG TGCAATATCC GGTCAAGAAA CAGAGCTTT CCGACAACCT
 3201 CGGCAAGGCA GAAGCCAAAA AACAGGGGGA AAAAGACACG CGGCAAGGCC
 3251 TTGACGCGCT GATTGCGGCC GGGCGGATG CCGTGGAAAA GACAGAAAGC
 3301 GTTGCCGACG CGGCGCGGCA GGCAGCGGGG GAAATGTGCG GCATTATGCA
 3351 GCGCGAGGAA GAGAAAAAAC GGGTGCGAGG GGATAAGAGC ACCGCTTTGG
 3401 CGAAACAGCG CGAAGCGGAA ACCCGGCGCT CTACACCGC CTTCGCCGCG
 3451 GCGCGCGCGC CGGCGCGGGA TACGCGCTTA TCGCAATAGC GATTAGGTAG
 3501 CGACGCGCAG CGGACCTGTA TCGCCGCTTA TCGCAATAGC GATTAGGTAG
 3551 AATTTCGCGC CAGCTCAAC AGGCTTTTGG CCGTACAGGA CGAATTAGAC
 3601 CGCGTATTGG CCGAAGACCG CGGCAACGCG GTTTGGACAA CGGCGATCCG
 3651 GGACACCAAA CACTACGTTT CGCAAGATTT CCGCGGCTAC CGCAACAAA

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3701 CCGACCTGCG CCAATCGGT ATGCAGAAA ACCTCGGCAG CGGCGCGTC
3751 GGCATCTGT TTTGCACAA CGGACGGAA AACACTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCACGGGCG CGTTTCGGG CAATACGGCA
3851 TCACAGGATT TCACATCGGC ATCAGCGCG GCSCGGGTTT TAGCAGCGGC
3901 AGCTTTTAC AGCGCATCGG AGGCAAAATC CGSCCGGCG TCGTCATTA
3951 CGCATTCAG GCACGATACC GCSCGGGTTT CGSCGGATTG GGCATGAAC
4001 CGCACATCGG CGCACGCGC TATTTCTCC AAAAAGCGGA TTACGCTAC
4051 GAAAACGTCA ATATCGCCAC CCCCgcgctt GCATTCAAC GCTACCGCG
4101 GGGCATTAAG GCAGATTATT CATTCAAAAC GCGCGAACAC ATTTCCATCA
4151 CGCCTTATT GAGCCTGTCC TATACGATG CCSCCTCGG CAAAGTCCGA
4201 ACACGCGTCA ATACCGCGT ATTGCTCAG GATTTCGGCA AAACCGCGAG
4251 TCGCGAATGG GCGCTAAACG CCGAAATCAA AGGTTTCAGC CTGTCCTTCC
4301 AGCTGCGCG CGCCAAAGGC CGCAACTGG AAGCGCAACA CAGCGCGGGC
4351 ATCAAATTAG GCTACCGCTG GTAA

```

This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1->:

```

orf1-1 .pep
1 MKTTDKRTE THRKAPKTYR IRFSPAYLAI CLSFGILPOA WAGHTYFGIN
51 YQYTRDPAEN KGRFAVGAOK IIRVNRKGL VGRSMTKAPM IDPSVVSRRG
101 VAALVYGDQYI VSVAHNGSYN NVDFGAERGM PDQHRFYIKI VKRNNYKACT
151 KGHYPGGDYH MRLRHKFTVD AEPVEMTSYM DGRKYIDQNN YDPRVRIAG
201 RQYWRSDDEDE PNNRESSYHI ASAYSWLVGG NTFAGNNGSG GTVNLGSEKI
251 KHSPYGFELPT GSGFGDSGSP MFYIDAKQKQ WLINGVLQTG NPYIKGKNGF
301 QLVRKDWFDYD EIFAGDTHSV FYEPFRQNGKY SFNDNNNGTG KINAKHEHNS
351 LPLNRKTRTV QLVNVLSET AREPVYHAAG GVNSYRPLRN NGENISFIDE
401 KRGELILTSN INQAGAGLYF QGDFTVSPEN NETWQAGVHV ISEDSVTWTK
451 VNGVANDRLS KIGKGLTHVQ ARGENGSGIS VGDGTVTLQD QADDGRKKQA
501 FSEIGLVSGR GTVQLAADNQ FNPDKLYEPE RGRGLDLNGH SLSEHRIQNT
551 DEGAMIVNHN QDKESTVTIT GNKDIATTGN NNSLDKKKEI AYNQWFGKED
601 TTKTNGRLNL VQPAEAERTL LLLSGGNTIN GNITQTNGKL FESGRPTPHA
651 YNHLNDHWSQ KEGIPRGEIV WDNWDINRTE KAENFQIKGG QAVSVNRVAK
701 VKGDWHLNHH AQAVFGVAPH QSHICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATINGNL SANGDTRYTV SHNATQNGNL
801 SLVGNQAQTF NQATLANGTS ASGNASPNLS DHAVONGSLT LSGNAKANVS
851 HSALAGNVSL ADKAVFHFES SRFTGDISGG KDTALHLKDS EWTLPSTGEL
901 GNHLNDWATI TLNSAYRIDA AGACTGSDAT APRRSRSRS RSLSVTPPT
951 SVESRENTLT VNGKLANGQT FRMSLEFGV RSKDKLAES SEGYTTLAVN
1001 NTGNEPASLE QLTVEGKDN KPLSENILFT LQNEHVDAGA WRYLIRKDG
1051 EFLRHNPKVE QELSDRLKA EAKQQAEDN AQSLDALIAA GRDAVEKTES
1101 VAEPARQAGG ENVGIMQAE EKKRVQADKO TALAKQREAE TRPATTAFPR
1151 ARRARRDLPO LQPOPOPOPO RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VMTSGIRDTR HYRSQDFRAY RQDTDLRIQ MQNLNLSGRV
1251 GLFSHNRTE NTFDDGIGNS ARLANGAVEG QYGDIDRFYIG ISAGAGFSSG
1301 SLSGIGGKI RRVRLAYGIG ARYRAGGFGF GIEPHIGATR YVQKADIRY
1351 ENVNIAITPGL AFNRYPAGIK ADYSPKPRQH ISTTPYLSLS YTDAAQKVR
1401 TRVNTAVLAQ DFKRTSSEW GVNAEIKRGT LSLHAAAAGK PQLAQHSAG
1451 IKLGYRW*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

```

orf46-2 .seq
1 TTGGGCATT CCGGCAAAAT ATCCCTTATT CTCTCCATAC TGGCAGTGTG
51 CCTGCCGATG CATGCACACG CCTCAGATTT GGCAAACGAT TCTTTTATCC
101 GGCAGGTTCCT GCACCGTCAG CATTTCGAAC CGCAGCGGAA ATACCACTTA
151 TTGGGCAGCA GGGGGGAAC TGGCGAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAATACAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGCGGCCCA
251 TTAAGGAAAA TATCGGCTAC ATTGTCGCTT TTTCGATCA CCGGACAGAA
301 GTCCATTCCC CTTTCAGCAA CATTCGCTCA CATTCCGATT CTGATGAAGC
351 CGGTAGTCCC GTTGACGGAT TTAGCCTTTA CGCATCCAT TGGACGGAT

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401 ACGAACACCA TCCGCGCAGC GGCTATGACG GGCCACAGGG CGGCGGCTAT
451 CCCGCTCCCA AAGCGCGCAG GGATATATAC AGCTACGACA TAAAGGCGGT
501 TGCCCCAAAT ATCCGCGCTCA ACCTGACGGA CAACCGCAGC ACCGGACAAC
551 GGCTTGCCGA CGGTTTCCAC AATGCCGATA GTATGCTGAC GCAAGGAGTA
601 GGCGAGCGAT TCAAAACGGC CACCCGATAC AGCCCGAGC TGGACAGATC
651 GGCCATAGCC GCCAAGCGCT TCAACGCGAC TGACAGATATC GTTAAAAACA
701 TCATCGCGCC GCGCAGGAGA ATGTGCGGCG CAGCGCTTGG GTCTGCTTTT
751 ATAAGCGAAG GCTCAAAACAT CTCTGTCATG CAGCGCTTGG GTCTGCTTTT
801 CACGAAACAC AAGATGGGCG GCATCAACGA TTTGGCAGAT ATGCGCGAAC
851 TCAAAAGACTA TGCCGCGAGA GCCATCCGCG ATTGGGCAGT CCAAACCGCC
901 AATGCCGCAC AAGGCATAGA AGCGCTCAGC AATATCTTTA TGGCAGCCAT
951 CCCCATCAAA GGGATTGGAG CTGTTCCGGG AAAATACGGC TTGGCGCGGA
1001 TCACGGCACA TCCTATCAAG CGGTCGCAGA TGGGCGCGAT CGCATTGCGG
1051 AAAGGGAATAT CGCGCGTCAG GCACATTTT GCGGATGCGG CATACGCCAA
1101 ATACCGCTCC CCTTACCAT CCGCAATAT CGGTTCAAA TGGAGCGAGC
1151 GTTACGGCAA AGAAACATC ACTCTCTAA CGGTGCGGCC GTCAAACGGC
1201 AAAAATGTCA AAGTGGCAGA CCAACGCCAC CGGTAAGACG GCGTACCGTT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGGCG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAACCAGAG ATGGGAGGTT GATAGGAAGC TTAATAAATT
1401 GACAACCTCG GAGCAGGTGG AGAAAAATGT TCAGGAATAA AGGAACGGTA
1451 ATATAACACG TAACTTTAGC CAACATGCTC AACTAGAGAG GGAATTAAT
1501 AAATAAAAT CTGCGCATGA AATTAATTT GCAGATGGAA TGGGAATTT
1551 TACCGATAGC ATGATGACA AGGCTTTTAG TAGGCTTTGG AAATCAGTTA
1601 AAGAGATATG CTTCACAAT CCAAGTGTGG ACTACGTTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AATAAATRGG GTTTTCTCTG CAGAAATCTT
1701 TGGCAGGATA CATGAATTTA AATTTAAAAA AGTTGACTTT CCGTTCTCTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGAATGAATC AGGTAATGTT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

orf46-2.pep

```

1 LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRQ HFEFDGKYHL
51 FGSRGELAER SGHILGLKIQ SHQLGNLMIQ QRAIKGNIGY IVRFSDHGHE
101 VHSFPDNHAS HSDSDEAGSP VDGFSLYRIH WDGVEHHPAD GYDGPQGGY
151 PAFKGARDIY SYDIKVAQN IRLNLTDRS TGORLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSGNA AEAFNGTADI VKNIIAAGE IVGAGDAVQF
251 ISBGSNIAM HGLGILLSTEN KMARINDLAD MAOLKDYAAA AIRDWAVQNP
301 NAAQGIKAVS NTFPMAPPIK GIGAVRGKIG LGGITAHPIK RSMQGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRHS LEQRYKEMI TSTVPKSN
401 KNVKLADQRH PKTGVPFDGK GFNFKEHKVH YDKLDOIQL SGGGIPKAKP
451 VFDKPRWEV DRKLNKLTR EQVEKNVQEI RNMGINSNFS OHAQLEREIN
501 KLSKADEINF ADGMGKFTDS MNDKAFSLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNR VFAAEYLGRI HELKFKKVDF PVPNTSWKNP TDVLNESGNV
601 KRPRYSK*

```

Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

Oligonucleotides used for PCR

Table 1

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ORF	Primer	Sequence	Restriction sites
279	Forward	CGCGGATCCCATATG-TTGCCTGCAATCACGATT <SEQ ID 90>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTAGAACGGGCGGCAA <SEQ ID 91>	XhoI
519	Forward	CGCGGATCCCATATG-TTCAAATCCTTTGTCGTCA <SEQ ID 92>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTGGCGGTTTGTCTGC <SEQ ID 93>	XhoI
576	Forward	CGCGGATCCCATATG-GCCGCCCCCGCATCT <SEQ ID 94>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATTTACTTTTTGATGTCGAC <SEQ ID 95>	XhoI
919	Forward	CGCGGATCCCATATG-TGCCAAAGCAAGAGCATC <SEQ ID 96>	BamHI-NdeI
	Reverse	CCCGCTCGAG-CGGGCGGTATTCGGG <SEQ ID 97>	XhoI
121	Forward	CGCGGATCCCATATG-GAAACACAGCTTTACAT <SEQ ID 98>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATAATAATATCCCGCGCCC <SEQ ID 99>	XhoI
128	Forward	CGCGGATCCCATATG-ACTGACAACGCACT <SEQ ID 100>	BamHI-NdeI
	Reverse	CCCGCTCGAG-GACCGCGTTGTCGAAA <SEQ ID 101>	XhoI
206	Forward	CGCGGATCCCATATG-AAACACCGCCAACCGA <SEQ ID 102>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTCTGTAAAAAAGATATGTGC <SEQ ID 103>	XhoI
287	Forward	CCGGAATCTAGCTAGC-CTTTCAGCCTGCGGG <SEQ ID 104>	EcoRI-NheI
	Reverse	CCCGCTCGAG-ATCCTGCTCTTTTGTGCC <SEQ ID 105>	XhoI
406	Forward	CGCGGATCCCATATG-TGCGGGACACTGACAG <SEQ ID 106>	BamHI-NdeI
	Reverse	CCCGCTCGAG-AGGTTGCTCTTGTCTATG <SEQ ID 107>	XhoI

EXAMPLE 2

Expression of ORF 919

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene 919 was cloned in pET vector and expressed in *E. coli*. The product of

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protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 3

Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 4

Expression of ORF 576

The primer described in Table 1 for ORF 576 was used to locate and clone ORF 576. The predicted gene 576 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B).. These experiments confirm that ORF 576 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 576 are provided in Figure 12. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 576 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 5

Expression of ORF 519

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein

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and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 6

Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene 121 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 7

Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene 128 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

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Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 8

Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

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antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 9

Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 10

Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*

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meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example 1.

The foregoing examples are intended to illustrate but not to limit the invention.

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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.

11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.

12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

15. Nucleic acid encoding a protein according to any one of claims 6-8.

16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.

17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.

19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.

20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.

21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.

22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.

23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.

24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.



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<p>Figure 1: Purification and Western blot analysis of Neisseria genomic sequences. Panel A shows a gel image of a purification step with lanes labeled "Purification" and "MI 919". Panel B shows a Western blot with lanes labeled "Western Blot", "OMV", "TP", and "PP". Panel C is a histogram showing the distribution of sequences, with a peak at 10^3. Panel D is a line graph showing the bactericidal assay results for preimmune, GST, and 919 sequences over time.</p>			
(57) Abstract			
<p>The invention provides methods of obtaining immunogenic proteins from genomic sequences including <i>Neisseria</i>, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of <i>Neisseria meningitidis</i> B. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.</p>			

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FIG. 1A

919 (46 kDa)

PURIFICATION

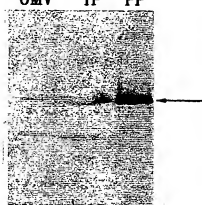
M1 919

*FIG. 1B*

919 (46 kDa)

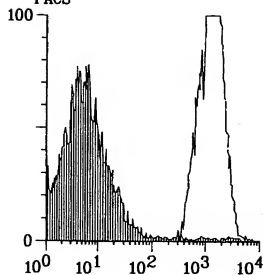
WESTERN BLOT

OMV TP PP

*FIG. 1C*

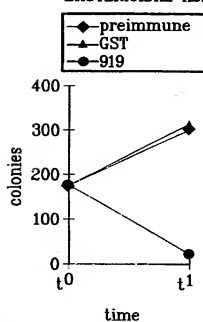
919 (46 kDa)

FACS

*FIG. 1D*

919 (46 kDa)

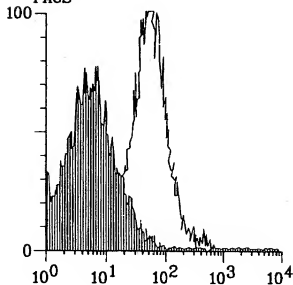
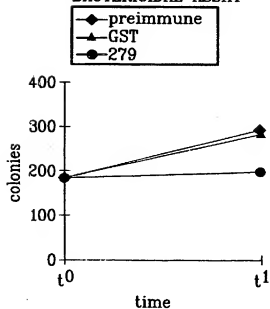
BACTERICIDAL ASSAY

*FIG. 1E*

919 (46 kDa)

ELISA assay: positive

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*FIG. 2A*279 (10.5 kDa)
PURIFICATION
M1 279*FIG. 2B*279 (10.5 kDa)
WESTERN BLOT
TP OMV*FIG. 2C*279 (10.5 kDa)
FACS*FIG. 2D*279 (10.5 kDa)
BACTERICIDAL ASSAY*FIG. 2E*

279 (10.5 kDa)

ELISA assay: positive

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FIG. 3A

576 (27.8 kDa)

PURIFICATION

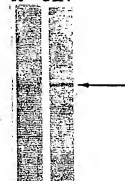
M1 576

*FIG. 3B*

576 (27.8 kDa)

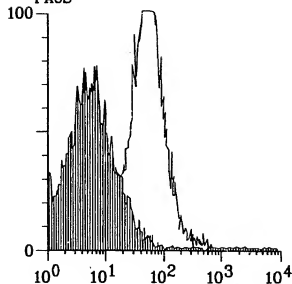
WESTERN BLOT

TP OMV

*FIG. 3C*

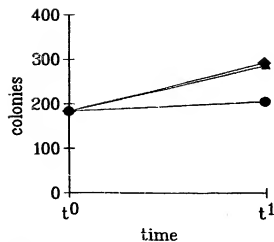
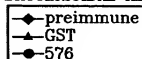
576 (27.8 kDa)

FACS

*FIG. 3D*

576 (27.8 kDa)

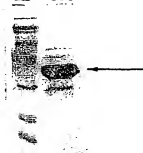
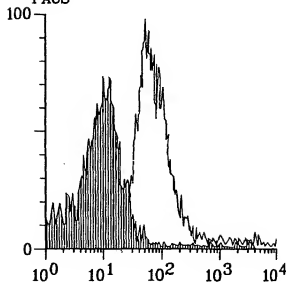
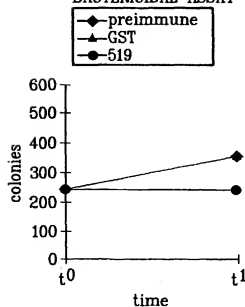
BACTERICIDAL ASSAY

*FIG. 3E*

576 (27.8 kDa)

ELISA assay: positive

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*FIG. 4A*519 (33 kDa)
PURIFICATION
M1 519*FIG. 4B*519 (33 kDa)
WESTERN BLOT
TP OMV*FIG. 4C*519 (33 kDa)
FACS*FIG. 4D*519 (33 kDa)
BACTERICIDAL ASSAY*FIG. 4E*519 (33 kDa)
ELISA assay: positive

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FIG. 5A

121 (40 kDa)
PURIFICATION
M1 121



FIG. 5B

121 (40 kDa)
WESTERN BLOT
TP OMV



FIG. 5C

121 (40 kDa)
FACS

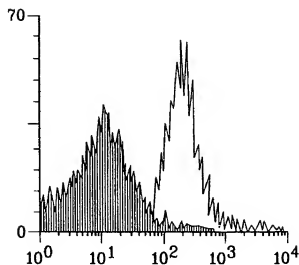


FIG. 5D

121 (40 kDa)
BACTERICIDAL ASSAY

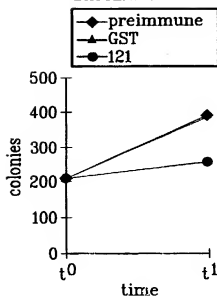


FIG. 5E

121 (40 kDa)

ELISA assay: positive

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FIG. 6A

128 (101 kDa)
PURIFICATION
M1 128



FIG. 6B

128 (101 kDa)
WESTERN BLOT
TP OMV

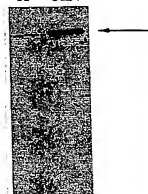


FIG. 6C

128 (101 kDa)
FACS

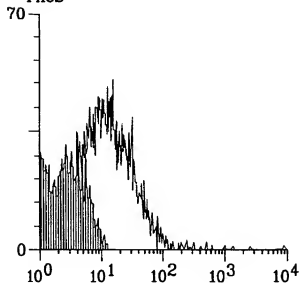


FIG. 6D

128 (101 kDa)
BACTERICIDAL ASSAY

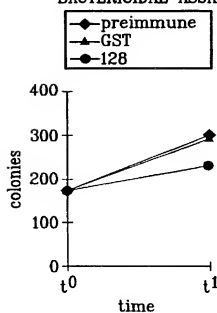


FIG. 6E

128 (101 kDa)
ELISA assay: positive

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FIG. 7A

206 (17 kDa)

PURIFICATION

M1 206

*FIG. 7B*

206 (17 kDa)

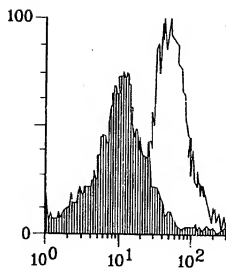
WESTERN BLOT

TP OMV

*FIG. 7C*

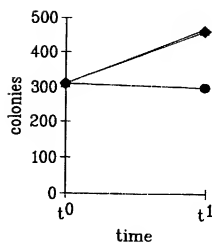
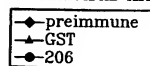
206 (17 kDa)

FACS

*FIG. 7D*

206 (17 kDa)

BACTERICIDAL ASSAY

*FIG. 7E*

206 (17 kDa)

ELISA assay: positive

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FIG. 8A

287 (78 kDa)

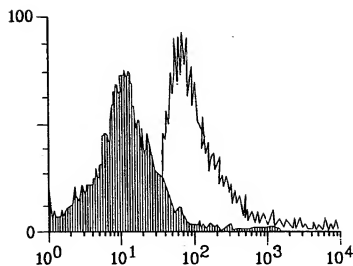
PURIFICATION

M1 287

*FIG. 8B*

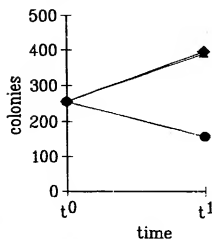
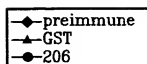
287 (78 kDa)

FACS

*FIG. 8C*

287 (78 kDa)

BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)

ELISA assay: positive

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FIG. 9A

406 (33 kDa)
PURIFICATION
M1 406



FIG. 9B

406 (33 kDa)
WESTERN BLOT
TP OMV



FIG. 9C

406 (33 kDa)
FACS

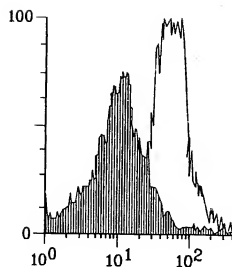


FIG. 9D

406 (33 kDa)
BACTERICIDAL ASSAY

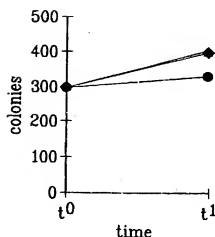
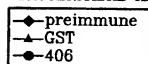


FIG. 9E

406 (33 kDa)

ELISA assay: positive

WO 00/66791

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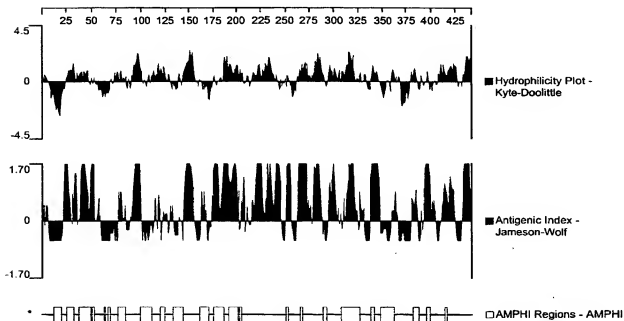
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 10

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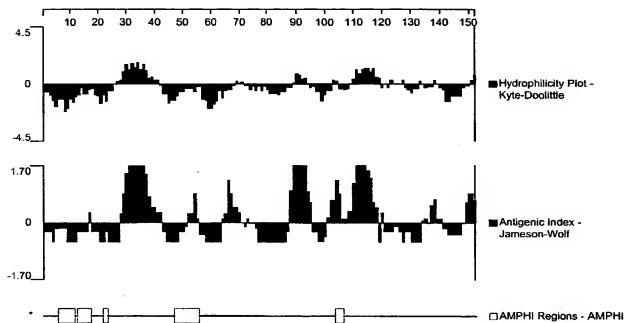
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

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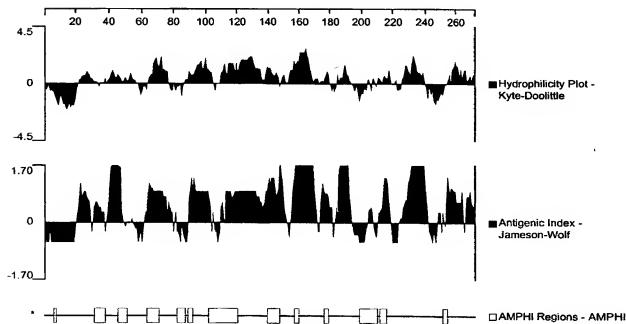
576-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 12

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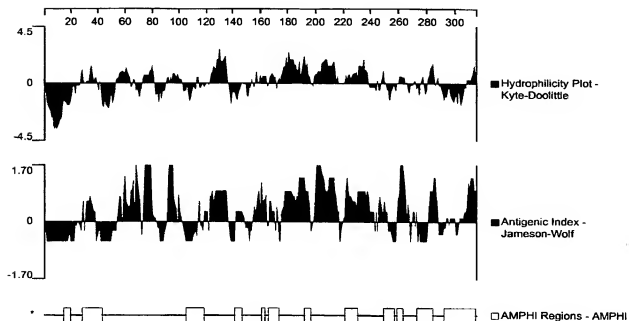
519-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 13

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121-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

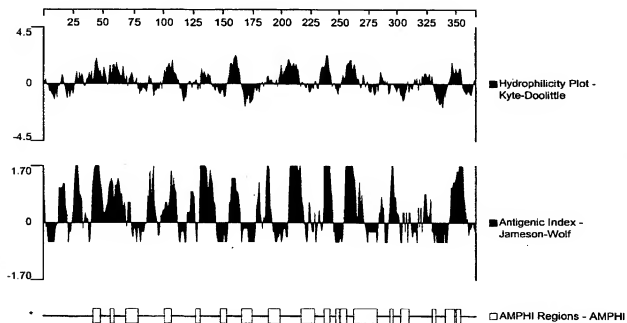


Fig. 14

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128-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

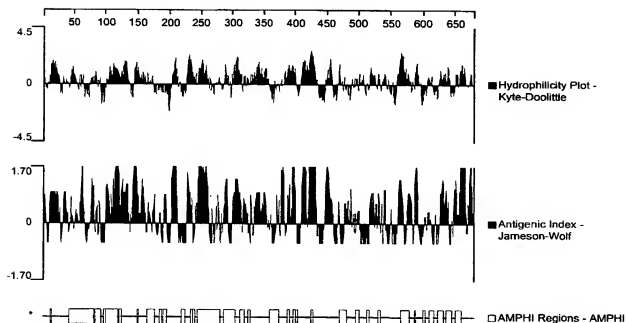


Fig. 15

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Hydrophilicity Plot, Antigenic Index and AMPHI Regions

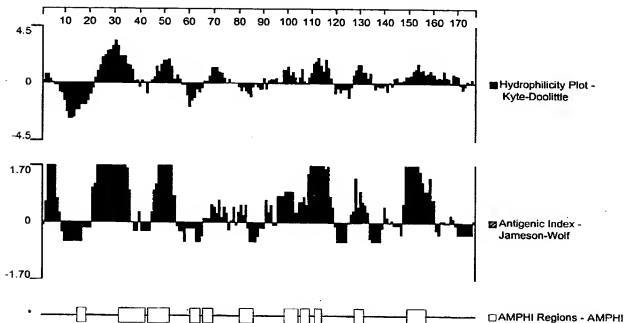


Fig. 16

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Hydrophilicity Plot, Antigenic Index and AMPHI Regions

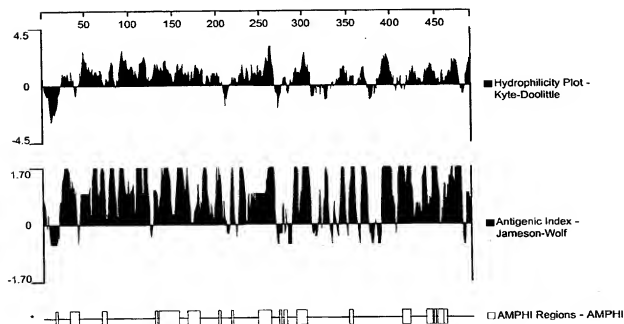


Fig. 17

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406

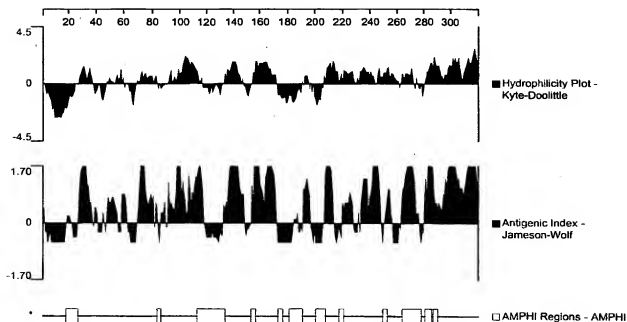
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 18

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

the specification of which (check one) ☐ is attached hereto ☒ was filed on March 8, 2000, as International Application No. PCT/US00/05928, and was amended on ☐ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)		Day/Month/Year Filed	Priority Claimed	
Number	Country		Yes	No
US00/05928	PCT	March 8, 2000	X	
0004695.3	GB	Feb. 28, 2000	X	
US99/23573	PCT	Oct. 8, 1999	X	

I hereby claim the benefit under Title 35, United States Code, §120 and/or §119(e) of any United States application(s) and/or provisional applications listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status Patented, Pending, Abandoned
60/132,068	April 30, 1999	Abandoned

101 Rec'd PCT/PTO 21 NOV 2002

PATENT
Atty Dkt. No. PP00365.322

#4

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" on **November 21, 2002** and addressed to: BOX PCT, Assistant Commissioner of Patent and Trademarks, Washington, D.C. 20231.

Mike Huser
Gyne Riser

11.21.02
Date

10/018470

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: **Pizza, M., et al.**

U.S. Serial No.: **10/018,470**

Int'l. Appln. No.: **PCT/US00/05928**

Group Art Unit: **not yet assigned**

Int. Filing Date: **March 8, 2000**

Examiner: **not yet assigned**

For: **NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE**

POWER OF ATTORNEY

Assistant Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. §1.36, Chiron Corporation, the Assignee of the above-identified patent application hereby appoints the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith.

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Dated: 11/21/02

CHIRON CORPORATION

By:

Alisa A. Harbin
Assistant Secretary

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PATENT
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Atty. Docket No. PP00365.322

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 Intl. App. No. PCT/US00/05928
 Atty. Docket No. PP00365.322

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Intl. App. No. PCT/US00/05928
Atty. Docket No. PP00365.322

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10/018470

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531 Rec'd PCT/PTC 30 OCT 2001

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